24/e

VOL. 40, PART 2 JUNE 1957

The British Mycological Society

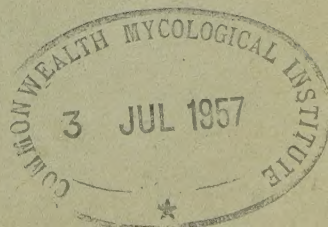
(Recognosce notum, ignotum inspice)

TRANSACTIONS

Edited by

G. C. AINSWORTH and S. D. GARRETT

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W.1
AMERICAN BRANCH: 32 EAST 57TH STREET,
NEW YORK 22, N.Y.



OFFICERS AND COUNCIL FOR 1957

President

C. J. HICKMAN, B.Sc., M.Sc., Ph.D.

Vice Presidents

L. OGILVIE, M.A., M.Sc.
W. D. GRADDON, B.Sc., A.M.I.Chem.E.
Miss L. E. HAWKER, D.Sc.

Secretary

J. WEBSTER, B.Sc., Ph.D.
Department of Botany, The University, Sheffield, 10.

Foray Secretary

Mrs S. M. FRANCIS, Ph.D.
Department of Botany, Imperial College of Science & Technology, Prince Consort Road, London, S.W. 7

Plant Pathology Committee Secretary

J. M. HIRST, D.S.C., B.Sc.
Rothamsted Experimental Station, Harpenden, Herts.

Treasurer

W. BUDDIN, M.A.
No. 1, Talfourd Avenue, Reading, Berks.

Editors

G. C. AINSWORTH, B.Sc., Ph.D., F.L.S.
Hatherly Laboratories, The University, Prince of Wales Road, Exeter, Devon

S. D. GARRETT, M.A., D.I.C., Sc.D.
Botany School, Downing Street, Cambridge

Council

The OFFICERS with the following in addition:

Miss A. H. S. BROWN	Miss J. FRAYMOUTH	P. H. GREGORY
R. V. HARRIS	D. M. HENDERSON	C. T. INGOLD
D. A. REID	J. T. SAVORY	T. J. WALLACE

B. BARNES, W. BROWN, A. D. COTTON, Dame HELEN GWYNNE-VAUGHAN, J. RAMSBOTTOM, E. W. SWANTON, Miss E. M. WAKEFIELD, M. WILSON

Plant Pathology Committee

Chairman: R. V. HARRIS, D.Sc., A.R.C.S.

E. W. BUXTON	R. J. W. BYRDE	F. C. DEIGHTON
D. J. GRIFFITHS	D. C. GWYNNE	Miss J. IVES
H. OWEN	D. RUDD-JONES	

The PRESIDENT, the SECRETARY and the PLANT PATHOLOGY COMMITTEE SECRETARY *ex officio*

Foray Committee

J. G. MANNERS D. A. REID Miss G. M. WATERHOUSE

The FORAY SECRETARY and the SECRETARY *ex officio*

The subscription price is 60*s.* per volume (post free), or $10.00 in U.S.A. payable in advance. Subscriptions may be sent to any Bookseller, or to the Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1.

Enquiries from the U.S.A. should be addressed to the Cambridge University Press American Branch, 32 East 57th Street, New York 22.

Trans. Brit. mycol. Soc. **40** (2), 177–186 (1957).

PLEOSPORA STRAMINIS, *P. RUBELLOIDES* AND *P. RUBICUNDA*: THREE FUNGI CAUSING PURPLE-STAINING OF DECAYING TISSUES

By JOHN WEBSTER

Botany Department, University of Sheffield

(With 3 Text-figures)

Pleospora straminis, previously unrecorded for Britain is described. *P. rubelloides* comb.nov. is described and compared with the related *P. rubicunda* on the host and in culture.

Herbaceous and woody debris decaying in damp situations, frequently shows purple staining. Various fungi such as *Leptosphaeria* spp., *Ophiobolus rubellus* and *Lophiotrema* sp. are sometimes present, but three species of *Pleospora* have been found associated with the purple stain. Since there has been some confusion in the nomenclature of these fungi, they are described below:

Pleospora straminis Saccardo, *Michelia*, **1**, 407, 1879 (Fig. 1)

$=$*P. obliqua* Wehmeyer, *Mycologia*, **43**, 579, 1951.

Several collections of this fungus on grasses, are in the Sheffield Herbarium:

Herb. Sheffield number	Host	Locality	Date
225	Wheat stubble	Londesborough, nr. Market Weighton, Yorks	26. vi. 49
226	Barley stubble	King's Lynn, Norfolk	7. viii. 49
227	Barley stubble	King's Lynn, Norfolk	7. viii. 49
821	*Dactylis glomerata*	Lindrick Common, nr. Worksop, Notts	15. vii. 50
1046	Wheat stubble	Bolton Percy, York. (coll. W. G. Bramley)	17. iii. 51

The following description is based on specimen 225 (see Fig. 1).

Perithecia, single beneath leaf sheath or stem, associated with a purple coloration; black, thin-walled, globose, surrounded at the base by sparse black hyphae, neck papillate, projecting; 240–390μ in diameter. *Asci*, broadly club-shaped, rounded at the tip, tapering to a short stalk below, bitunicate, 3–8 spored, 130–192 $\times$ 23–32μ interspersed by branched filiform 'paraphyses'.

Ascospores, biseriate in the upper portion of the ascus, uniseriate below, oblong-elliptical with rounded ends, the upper half of the spore larger than the lower, with nine to eleven transverse septa, constricted at the septa,

Vol. 40, Part 1 was issued 25 March 1957

especially at the middle, and with numerous longitudinal septa; golden-yellow in colour, brown with age; 26–34 × 10–12 μ.

Cultures. On potato-dextrose agar the fungus grew slowly (2·5 cm. in 6 weeks at room temperature), producing a pink colour in the medium, the colonies becoming black at the margins. Perithecia were formed in 4 weeks in cultures started from either single ascospores or asci, and were

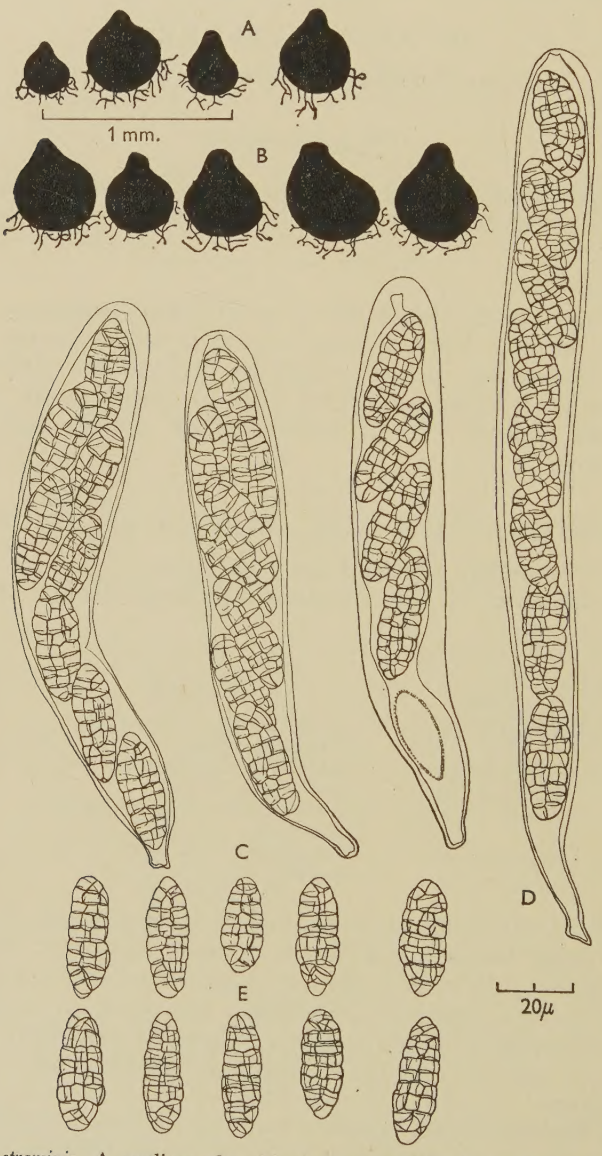


Fig. 1. *Pleospora straminis.* A, outlines of perithecia from stubble; B, outlines of perithecia from culture on P.D.A.; C, asci from the host; D, ascus from culture; E, ascospores from the host (Herb. Sheffield 225.).

frequently larger than those on stubble, measuring 330–450μ in diameter. The asci were also larger, 155–230 × 20–27μ.

For measurements of asci and ascospores from other collections see Table 1.

Wehmeyer (1951) has re-described *P. straminis* as *P. obliqua.* Material from specimen 255 was at first identified by him as *P. obliqua.* Later, having seen authentic material of *P. straminis* (which I had been unable to obtain), he agreed that specimen 255 was identical with *P. straminis.* There is a curious discrepancy in the measurements given for this fungus, however. Saccardo's measurements (see Table 1) are appreciably larger than those given by Berlese from the type, which match more closely those from the present collections.

Pleospora rubicunda Niessl., *Verh. naturforsch. Ver. Brünn,* **14**, 1876. (Fig. 2)

Several collections of this fungus have been made, mainly on various kinds of plant debris near the sea. The following description is based on Herb. Sheffield no. 366 on *Artemisia* (?), Wolferton Marshes, Norfolk, 6 August 1948.

Perithecia, purple to black in colour; seated on purple-stained areas of tissue, developing beneath the surface, later semi-erumpent with about half the perithecium exposed, subglobose or elongated parallel to the long axis of the host, up to 400μ in diameter; neck papillate, ostiole often slit-like; perithecial wall uneven in thickness, 40–50μ thick above, about 30μ thick below. *Asci,* bitunicate, numerous, broadly club-shaped to cylindrical, rounded above, tapering to a stalk below, up to 8-spored, 170–240 × 16–22μ; separated by numerous branched 'paraphyses'. *Ascospores,* biseriate in the broader portion of the ascus, uniseriate below, crescent-shaped when viewed laterally, with 9–13 transverse septa and one or more longitudinal septa in the middle cells, tapering and rounded at the ends, constricted at the septa, especially in the middle; yellow to brown in colour, 30–40 × 8–12μ.

Cultures. Ascospores germinated readily on agar media producing germ tubes, apparently from any cell. Cultures started from single ascospores and single asci were transferred to slopes of oat or potato-dextrose agar. The fungus grew slowly, forming colonies about 5 cm. in diameter in 2 months, with a characteristic purple colour in the medium and low pale aerial mycelium. Pycnidia of the *Phoma* type were found in 2-month-old cultures. The pycnidia were up to 500μ in diameter, subglobose, wall thin (10–15μ) black, composed of dark interwoven hyphae. The sporophores form a palisade of hyaline skittle-shaped cells lining the wall and abstrict spherical to broadly elliptical hyaline pycnospores about 2μ in diameter. Masses of pycnospores exude as flesh-coloured drops from the ostioles of the pycnidia. Perithecia have been found only once in culture (a multi-ascospore isolate of specimen 1723). When discovered, these perithecia were in poor condition and contained collapsed and distorted ascospores.

Pycnidia. Pycnidia resembling those found in culture have been found associated with perithecia. They are globose, submerged at first, later more

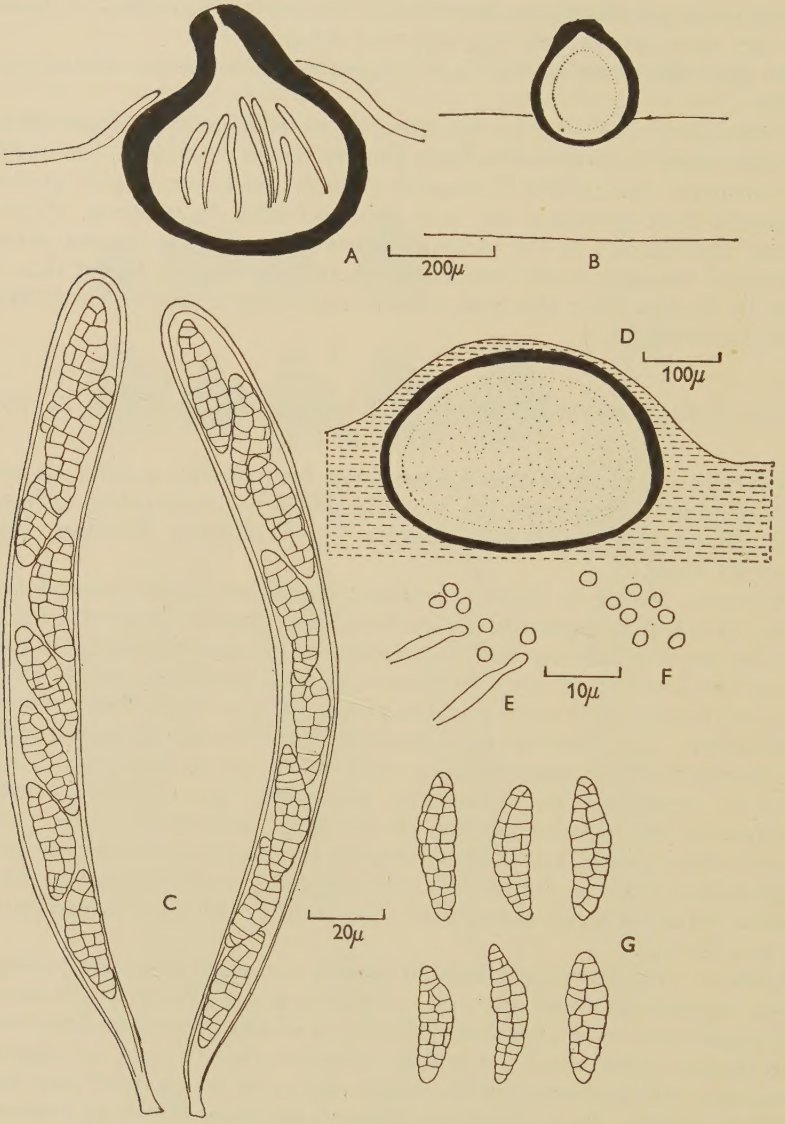


Fig. 2. *Pleospora rubicunda*. A, L.S. host stem and perithecium; B, L.S. host stem and pycnidium; C, asci from the host; D, section of culture showing pycnidium; E, pycnospores and sporophores from the host; F, pycnospores from culture; G, ascospores from the host. (A, C, D, G, from Herb. Sheffield 336; B, E, from Herb. Sheffield 1752B.)

superficial, up to 200μ in diameter, thin-walled and containing numerous spherical or elliptical pycnospores about 2μ in diameter. When pycnospores from the host are transferred to agar media, they germinate after about 1 week, giving rise to cultures which resemble those derived from ascospores.

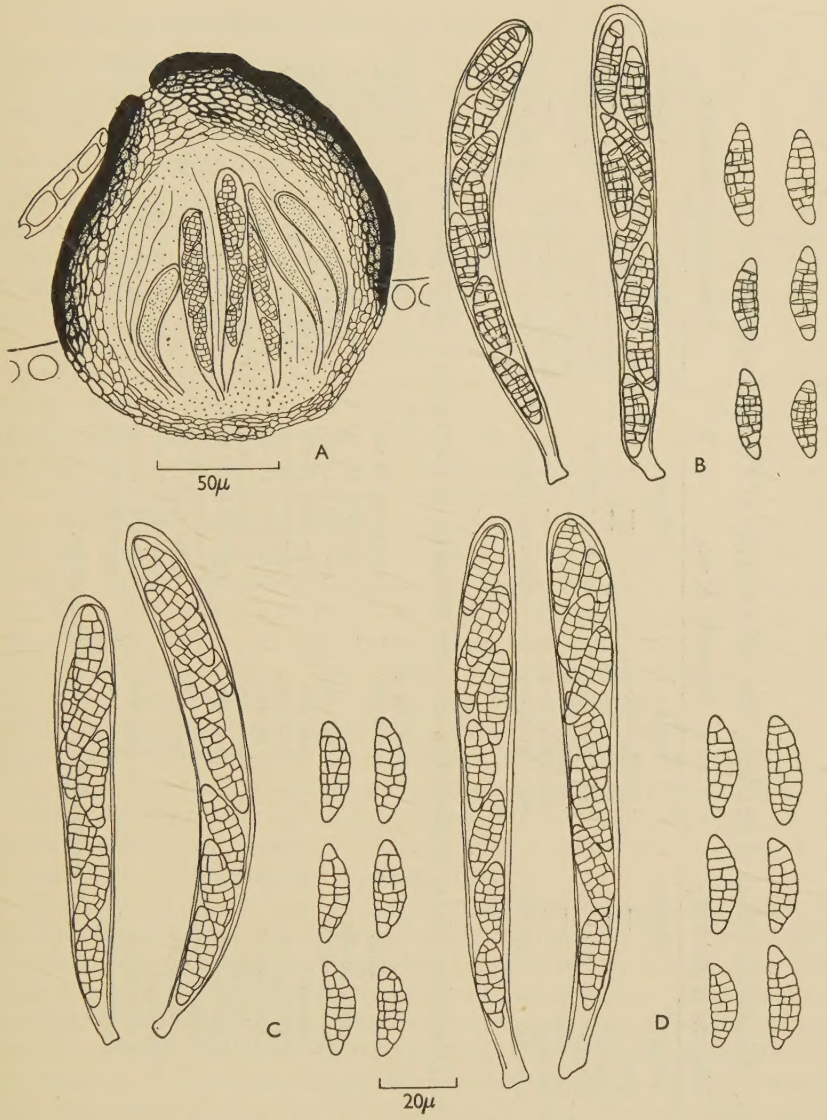


Fig. 3. *Pleospora rubelloides*. A, L.S. wheat stem and perithecium; B, asci and ascospores; C, asci and ascospores from the host; D, asci and ascospores from culture on oat agar. (A, C, D, from Herb. Sheffield 1722; B from the type specimen of *Sphaeria rubelloides*.)

Other collections. I have collected the fungus in Britain, France and Denmark only in maritime habitats, on the following hosts: *Artemisia* (?), *Lepidium latifolium* and *Halimione portulacoides*, and on various fragments of unidentified debris washed up by the sea, including woody plants. Niessl's original collection from Brünn was on *Sambucus ebulus*, but the fate of this specimen is not known. The dimensions of some recent collections and the type are listed in Table 2.

Table 1. *Dimensions (in μ) of perithecia, asci and ascospores of* Pleospora straminis

Specimen or authority	Diameter of perithecia		Dimension of asci		Dimensions of ascospores	
	Host	Culture	Host	Culture	Host	Culture
Saccardo, 1879	330	—	140–150 × 25–30	—	40–45 × 16–18	—
Type (Berlese, 1900)	300–350	—	120–130 × 24–28	—	34–38 × 12–14	—
Sheffield 225	240–390	330–450	130–192 × 23–32	155–230 × 20–27	26–34 × 10–12	24–37 × 9–12
Sheffield 226	360–460	—	120–190 × 20–26	—	30–41 × 11–13	—
Sheffield 227	180–250	—	120–170 × 20–24	—	29–37 × 10·5–12·5	—
Sheffield 821	240–260	350–650	104–140 × 20–27	100–190 × 20–26	25–34 × 10–14	30–36 × 11–13

Table 2. *Dimensions (in μ) of perithecia, asci and ascospores of* Pleospora rubicunda

Specimen or authority	Host	Diameter of perithecia	Dimensions of asci	Dimensions of ascospores	Transverse septa
Niessl, 1876	*Sambucus ebulus*	350	140–160 × 18–22	30–33 × 11–13	10–11
Type (Berlese, 1900)	*Sambucus ebulus*	250–350	110–130 × 14–18	26–32 × 8–10	12–15
Sheffield 366*	*Artemisia?*	300–400	170–240 × 16–22	30–40 × 8–12	9–13
Sheffield 855	Unidentified	250–320	130–200 × 18–20	29–36 × 9–11	7–10
Sheffield 856*	Unidentified	250–400	170–200 × 18–20	30–40 × 9–12	9–10
Sheffield 857	Unidentified	300–400	200 × 20–22	30–40 × 9·5–12	9–10
Sheffield 861	Unidentified	300–400	150–180 × 20–22	34–41 × 10–12	9–10
Sheffield 863	*Halimione portulacoides*	350	150–170 × 18–22	34–40 × 9–11	8–11
Sheffield 1674*	Unidentified	320–400	180–190 × 18–20	28–32 × 9–10	9–10
Sheffield 1723*	*Spartina?*	400	130–180 × 18–20	35–46 × 10–12	9–11
Sheffield 1727*	Unidentified	320–350	210–270 × 20–24	30–44 × 8–13	9–11
Sheffield 1752*	*Lepidium latifolium*	290–320	154–166 × 20–24	32–40 × 9–10	9–10

* Produced *Phoma* pycnidia in culture.

Pleospora rubelloides (Plowright ex Cooke) comb.nov. (Fig. 3)

=*Sphaeria rubelloides* Plowright ex M. C. Cooke, *Grevillea*, **5**, 120, 1877.
Leptosphaeria rubelloides (Plowright) Sacc., *Sylloge Fungorum*, **2**, 77, 1883.
Pleospora minor Wehmeyer, *Amer. J. Bot.* **39**, 242, 1952.

Cooke (1877) described this fungus as follows:

'*Sphaeria rubelloides*. Plow. Perithecia scattered, soon exposed, mostly seated on reddish stains; asci lanceolate, sporidia fusiform, multiseptate (about 9) yellow. On putrid culms of *Triticum repens*, King's Lynn (C.B.P.) Sporidia ·03 × ·007 mm.' Examination of Plowright's specimens in Herb. Kew. (on *Triticum repens*, Ap. 1876 Sea Bank, King's Lynn), showed that the fungus is a *Pleospora*. Unfortunately, since the description makes no mention of longitudinal septa, Saccardo (1883) compiled it as a *Leptosphaeria*. Berlese (1894) added support to this transfer by citing it as a synonym of *L. culmiphraga* (Fr.) Ces. & de Not. Wehmeyer (1952) has since redescribed the fungus under the name *Pleospora minor*. Curiously, his description is based on a later collection of Plowright's from King's Lynn, labelled *P. rubicunda* Niessl. Plowright evidently was confused between his *Sphaeria rubelloides* and *Pleospora rubicunda*. In 1877 Phillips & Plowright listed '*Sphaeria* (*Pleospora*) *rubicunda* Niessl. South Wootton. May, 1877. Sporidia 35–10 mk. On *Juncus*. Differing from *Sp. rubelloides*, Plow., *Grevillea*, Vol. v, p. 120, in the larger and muriform sporidia.' This specimen (Plowright Sph. Britt. 3, no. 70), however, matches the type material of *S. rubelloides*. A later specimen (Plowright Sph. Britt. 3, no. 71) labelled '*Sphaeria* (*Pl.*) *rubicunda* Niessl Lynn 1879, *Sp. rubelloides*, Plow. on putrid grass', also matches *S. rubelloides*. Collections by Plowright on rotten wood, Brandon, Norfolk, 1879, in Cooke's Herbarium at Kew are also of *S. rubelloides*, although labelled *Pleospora rubicunda*. *P. rubicunda* and *Sphaeria rubelloides* are, however, distinct, and in view of the confusion, the type specimen of *S. rubelloides* is redescribed.

Perithecia, black, up to 250μ in diameter, globose or somewhat elongated parallel to the stem, subepidermal, piercing the epidermis by a small papillate ostiole, later raising the epidermis and becoming almost superficial; seated on purple-coloured patches on the decaying stems. *Asci*, numerous, club-shaped, tapering at the base to a short stalk, rounded at the apex, bitunicate, usually 8-spored; 94–120 × 12–16μ. *Ascospores*, uniseriate in the lower portion of the ascus, irregularly biseriate above; yellow to brown in colour, crescent-shaped when viewed laterally, with one face flattened, the other sharply curved, tapering at the ends, with six to nine transverse septa (usually seven) and a variable number of longitudinal septa in all but the end cells; slightly constricted at the transverse septa, especially at the middle; 20–26 × 6–8μ.

Other collections. Over fifty other collections have been made on various hosts in Britain, France and Denmark. The fungus is common during the summer on old straw and grasses rotting under damp conditions, but is also found on other plant debris, especially in estuaries, salt marshes and sand dunes, along with the related *Pleospora rubicunda*. The fungus has been collected on the following hosts: *Agropyron repens*, *A. junceiforme*, *Ammophila*

arenaria, *Arctium minus*, *Dactylis glomerata*, *Elymus arenarius*, *Festuca arundinacea*, *Melilotus altissima*, *Scirpus maritimus* and on wheat and barley stubble. The range of variation is naturally greater than that found in the type specimen, and the dimensions of perithecia, asci, ascospores on the host and in culture are listed below (Table 3). In certain collections, the size of the ascospores is appreciably greater than in the type collection (e.g. 26–34 × 7–10μ in specimen 212, and 26–36 × 9–11μ in specimen 1743). The number of transverse septa may also be greater. However, the size of ascospores obtained in cultures from such collections, approximates more closely to that of the type.

Cultures. Ascospores transferred to agar media germinate overnight, producing a germ tube from any cell. The fungus is homothallic and forms perithecia readily within 2 months on oat agar slopes. The characteristic purple coloration is formed in the medium as a secretion of the hyphae, which are colourless. A low-growing felt of white aerial mycelium covers the surface of the medium and the perithecia, which are formed within 2 months, are frequently in contact with the glass of the culture vessel. The perithecia are globose with a short blunt neck, and may be larger than those found on the host, measuring up to 500μ. Similarly, the asci may be longer (up to 180μ in culture, compared with up to 140μ on the host). The dimensions of the ascospores formed in culture show less variation, however, than those on the host. No other type of fructification has been seen in cultures of *P. rubelloides*.

Discussion

The three fungi described above are clearly related to each other. Berlese (1888) pointed out that 'La *Pleospora rubicunda* è affine alla *Pleospora straminis*'. *P. rubicunda* and *P. rubelloides* are, perhaps, more closely similar. Whilst most collections of *P. rubicunda* can generally be separated from the majority of collections of *P. rubelloides* by the larger perithecia, longer and wider asci, and larger ascospores with more numerous transverse septa, none of these characters is completely reliable due to the overlap in the range of dimensions in the two species. Indeed, certain collections here assigned to *P. rubelloides* (e.g. 212 on *Agropyron repens* and 1743 on *Ammophila arenaria*) with rather large spores or wide asci, have been determined only after recourse to cultures. The absence of a *Phoma* stage in culture and the production of perithecia in single ascospore cultures, have been taken as evidence supporting this determination, and this is further supported by the more normal size of ascospores in culture in specimen 212. The existence of such 'intermediate' collections raises the question whether we should consider the two 'species' as a single more variable one. At present the only reliable criterion separating the two species is the production of a '*Phoma*' stage in cultures assigned to *P. rubicunda*. Perithecia have been found only once in *P. rubicunda* in a multi-ascospore culture, and have never been observed in cultures derived from single ascospores, whilst in *P. rubelloides* perithecia are formed readily in single ascospore cultures. This may imply that whilst *P. rubelloides* is homothallic, *P. rubicunda* is heterothallic, but the evidence on this point is not completely satisfactory.

Table 3. *Dimensions (in μ) of perithecia, asci and ascospores of* Pleospora rubelloides

Specimen	Host	Diameter of perithecia		Dimensions of asci		Dimensions of spores		Transverse septa	
		Host	Culture	Host	Culture	Host	Culture	Host	Culture
Type	*Agropyron repens*	250	—	94–120 × 12–16	—	20–26 × 6–8	—	6–9	—
Sph. Britt. III, 71	Putrid grass	250	—	116–130 × 17–21	—	24–33 × 7·5–10	—	8–10	—
Herb. M. C. Cooke	Rotten wood (Brandon)	200–300	—	86–130 × 13–17	—	22–28 × 6–8	—	7–9	—
Sheffield 212	*Agropyron repens*	200–320	300–350	98–126 × 15–19	130–180 × 12–16	26–34 × 7–10	22–27 × 6·5–8	8–12	7–9
Sheffield 850	*A. repens*	200–240	300–500	86–108 × 16–17	106–112 × 14–15	23–28 × 7–8	22–28 × 6·5–9	7–9	7–9
Sheffield 853	*A. repens*	160–200	300–400	80–90 × 14–16	114–120 × 16–18	20–22 × 7	22–30 × 7–10	7	7–9
Sheffield 1722	Wheat straw	200–250	200–350	94–120 × 15–16	120–150 × 13–18	20–26 × 6·5–8	22–30 × 6·5–9	6–8	7–9
Sheffield 1739	*Festuca arundinacea*	200–240	200–380	120–140 × 15–16	124–170 × 14–17	22–25 × 7·5–8	22–26 × 6·5–8	7–9	7–8
Sheffield 1740	*Melilotus altissima*	200–250	250	120–130 × 14–16	130–140 × 13–15	24–28 × 7–9	22–25 × 7–8	7–8	7–9
Sheffield 1743	*Ammophila arenaria*	250–300	200–290	110–150 × 20–24	120–140 × 20–22	26–36 × 9–11	27–34 × 8·5–11	8–10	8–10
Sheffield 1748	*Scirpus maritimus*	200–250	250–350	100–120 × 14–16	130–160 × 14–16	22–28 × 7–8	25–30 × 8–10	7–9	7–8
Sheffield 1749	*Elymus arenarius*	200–250	200–300	100–115 × 15–16	110–150 × 14–18	22–28 × 7–8	27–32 × 8–10	7–9	7–9
Sheffield 1750	*Festuca arundinacea*	280–350	250–300	100–114 × 14–16	120–160 × 14–16	21–28 × 6·5–7	24–32 × 8–9	7–9	7–9
Sheffield 1751	Barley straw	250–300	200–250	100–110 × 13–16	130–140 × 14–18	20–26 × 7–8	26–32 × 7–9	7–8	7–9

I am grateful to the Director of the Royal Botanic Gardens, Kew, for permission to study specimens, and to Dr R. W. G. Dennis for helpful discussion. To Prof. L. E. Wehmeyer my thanks are due for examining certain collections. The work has been assisted by a grant from the University of Sheffield Research Fund.

REFERENCES

Berlese, A. N. (1888). Monografia dei generi *Pleospora*, *Clathrospora* and *Pyrenophora*. *Nuovo Giorn. Bot. Ital.* **20**, 5–176.

Berlese, A. N. (1894). *Icones fungorum omnium hucusque cognitorum*, **1**.

Berlese, A. N. (1900). *Icones fungorum omnium hucusque cognitorum*, **2**.

Cooke, M. C. (1877). New British Fungi. *Grevillea*, **5**, 118–122.

Niessl, G. (1876). Notizen über neue und kritische Pyrenomyceten. *Verh. naturf. ver. Brünn.* **14**, 165–208.

Phillips, W. & Plowright, C. B. (1877). New and rare British fungi. *Grevillea*, **6**, 22–29.

Saccardo, P. A. (1879). Fungi veneti novi vel critici vel Mycologiae Veneti addendi. IX. *Michelia*, **1**, 359–445.

Saccardo, P. A. (1883). *Sylloge Fungorum omnium hucusque cognitorum*, **2**.

Wehmeyer, L. E. (1951). Studies in the genus *Pleospora*. IV. *Mycologia*, **43**, 570–589.

Wehmeyer, L. E. (1952). Studies in the genus *Pleospora*. VI. *Amer. J. Bot.* **39**, 237–243.

(*Accepted for publication* 21 *February* 1956)

Trans. Brit. mycol. Soc. **40** (2), 187–192 (1957).

PENICILLIUM ISARIAEFORME SP.NOV. AND *ASPERGILLUS RAPERI* SP.NOV.

By AMELIA C. STOLK

Centraal Bureau voor Schimmelcultures, Baarn, Netherlands

and J. MEYER

Institut National pour l'Étude Agronomique du Congo Belge, Yangambi, Belgian Congo

(With Plate 8 and 2 Text-figures)

Two new fungi, isolated from soil of the Belgian Congo, are described and figured. *Penicillium isariaeforme* sp.nov. belongs to the *P. claviforme* series and is characterized by very large Isaria-like coremia. *Aspergillus raperi* sp.nov. belongs to the *A. versicolor* series and is characterized by conidial heads with one series of sterigmata and by globose bodies consisting only of hülle cells.

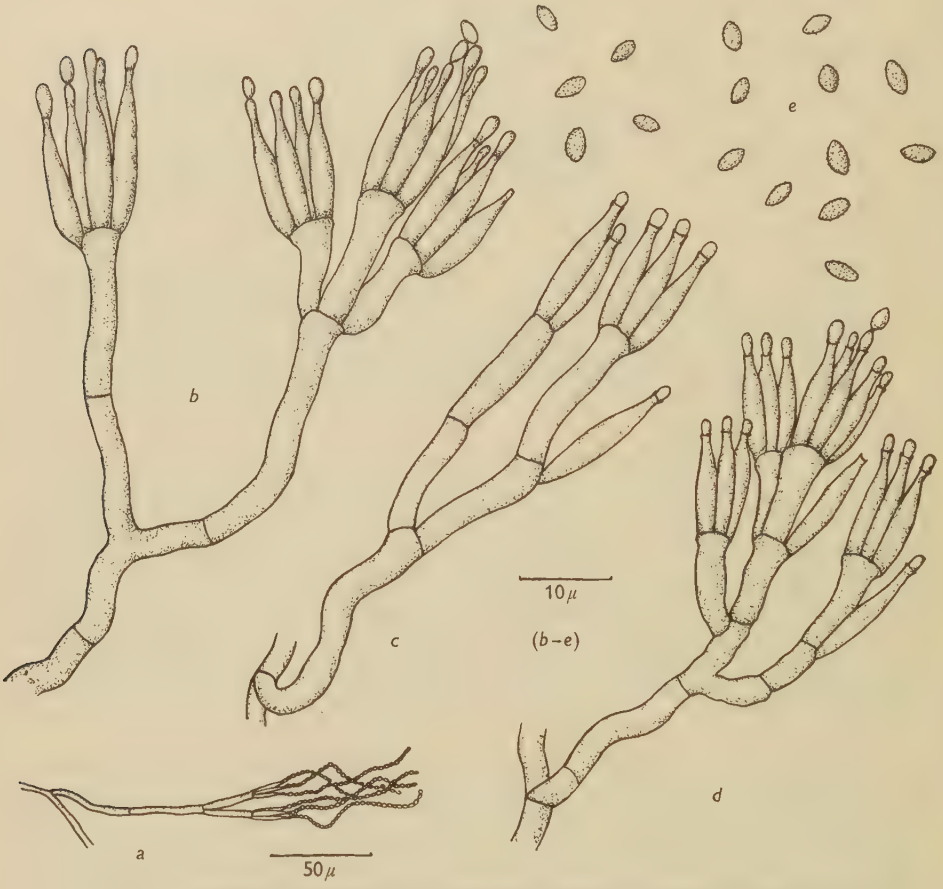
The two fungi described in this paper were isolated from soil of the Belgian Congo and sent to the Centraal Bureau voor Schimmelcultures (C.B.S.), Baarn, for identification. Both fungi proved to be different from all described species of *Penicillium* and *Aspergillus*. Prof. Dr Kenneth B. Raper, who very kindly examined both fungi, concurs in this opinion. Living cultures of the studied species are maintained at the C.B.S. and will be sent to the Commonwealth Mycological Institute, Kew, England. Dried cultures will be deposited in the Rijksherbarium, Leiden, Netherlands, and in the Herbarium of the Royal Botanic Gardens, Kew, England.

Penicillium isariaeforme sp.nov. (Pl. 8, figs. 1–3; Text-fig. 1)

Coloniae, in agaro Czapeki optime crescentes, consistunt e mycelio basali lente funicoloso, humiliter crescente, colore pallide flavo-viridi propter penicillus in eo sparse crescentes; coloniae saepe stellaeformes, coremia isariaeformia in zonis concentricis formata, plerumque non ramificata, usque 15 cm. longa in lagena 'Erlenmeyer' 1 L. continente, 1–4 mm. in diam., olivacea albis apicibus, saepe zonata, zonis sterilibus pallide flavis alternantibus cum zonis fertilibus olivaceis, positive phototropica; mycelio sterili albo et flavo; reversum coloniarum flavum; penicilli numerosi efficiunt stratum hymenii formam habens circa coremia; penicilli biverticillati, asymmetricales, variabiles, interdum uno aut duobus ramis lateralibus, interdum ex uno verticillo metularum compositi, interdum monoverticillati; conidiophora sinuata, parietibus glabris, 30–350 × 3–3·5 μ; rami laterales sinuati, glabri, 10–25 × 2·5–3 μ; metulae 2–4 in singulo verticillo, 9–15 × 3–3·5 μ, paululum divergentes, in apicibus tumidae; sterigmata 2–7 in singula metula, 12–16 × 2·5–3·5 μ, parallela, similia sterigmatibus Biverticillatae Symmetricae; conidia elliptica, utrimque acuta, 3–3·8 × 2–2·5 μ, quorum parietes minimis spinis sparsi sunt, in catenulis implicatis. Hab. in solo Congo Belgicae, apud pagum Yangambi, Africa, leg. J. Meyer, 1949.

Colonies on Czapek agar in Petri dishes grow well, attaining a diameter of about 4 cm. in 14 days at room temperature (20° C.). They are extremely

fasciculate, consisting of a fairly tough, somewhat funiculose but essentially plane basal felt from which the Isaria-like coremia arise in conspicuous, concentric zones. The basal felt, generally somewhat raised in central areas, bears loosely scattered, separate penicilli in pale yellow green shades, near Dull Green Yellow (Ridgway, 1912) and Deep Sea-foam Green,



Text-fig. 1. *Penicillium isariaeforme*. *a*, penicillus producing conidia in tangled chains; *b–d*, penicilli of different types; *e*, conidia.

becoming Drab in age. The colony margin is in very young cultures even with advancing zone white and somewhat fibrous owing to developing coremia. Older colonies often become star-like on account of the growth of the exterior coremia (Pl. 8, fig. 1). The coremia are fibrous, mostly simple, 1–4 mm. in diameter throughout and up to 15 cm. long in a 1 l. Erlenmeyer flask. The coremia are not differentiated into a stalk and a spore-bearing tip, they are at first white to Light Chalcedony Yellow, but soon develop yellow green shades near Light Hellebore Green and Cress Green becoming Jade Green or Olive in age. Growing coremia are charac-

terized by conspicuous white tips (Pl. 8, fig. 2). Often the stalks are zonate, fertile green bands alternating with sterile yellow ones (Pl. 8, fig. 3). The coremia are strongly positively phototropic. Areas of flocculent, sterile, white or pale yellow overgrowth may develop in older cultures, thus altering the cultural aspect of the colony. This occurs especially in tubes and in Erlenmeyer flasks containing a deep layer of culture medium. Exudate in pale yellow drops. Odour mouldy. Reverse in yellow shades, near Light Chalcedony Yellow, beneath the coremia Light Grayish Olive and in the centrum becoming Deep Olive-buff in age. The penicilli are abundantly produced along the coremia, sometimes individually hardly distinguishable, with elements closely crowded and interlaced, forming a hymenium-like spore-bearing surface. The *penicilli* (Text-fig. 1*b–d*) are mostly composed of well-differentiated—but irregular—asymmetric structures, showing usually one or more sinuous, divergent branches on different levels in addition to the main axis, or bearing only a single terminal verticil of metulae. Secondary penicilli arising from branches are often monoverticillate. *Conidiophores* sinuous, with smooth walls, mostly short about 30–100μ in length, but sometimes up to 350μ, and 3–4μ in diameter. Branches sinuous, smooth, 10–25 × 2·5–3μ. *Metulae* usually in groups of two to four, measuring 9–15 × 3–3·5μ, somewhat divergent, inflated at the apices. *Sterigmata* few to the metula, commonly in groups of two to seven, conspicuously parallel, giving the surface of the coremia its palisade-like appearance, 12–16 × 2·5–3·5μ, of an almost constant diameter throughout, gradually tapering into conidium-bearing tips in a manner suggestive of the Biverticillata-Symmetrica. Conidium-bearing tubes of the sterigmata cup-like, thus designating the sterigmata as phialides. *Conidia* elliptical, with one or both ends pointed, 3–3·8 × 2–2·5μ, with walls delicately spinulose (Text-fig. 1*e*), abundantly produced in tangled chains (Text-fig. 1*a*).

Colonies on malt agar show a conspicuous tendency to develop areas of flocculent, sterile overgrowth, coremia and penicilli as described above.

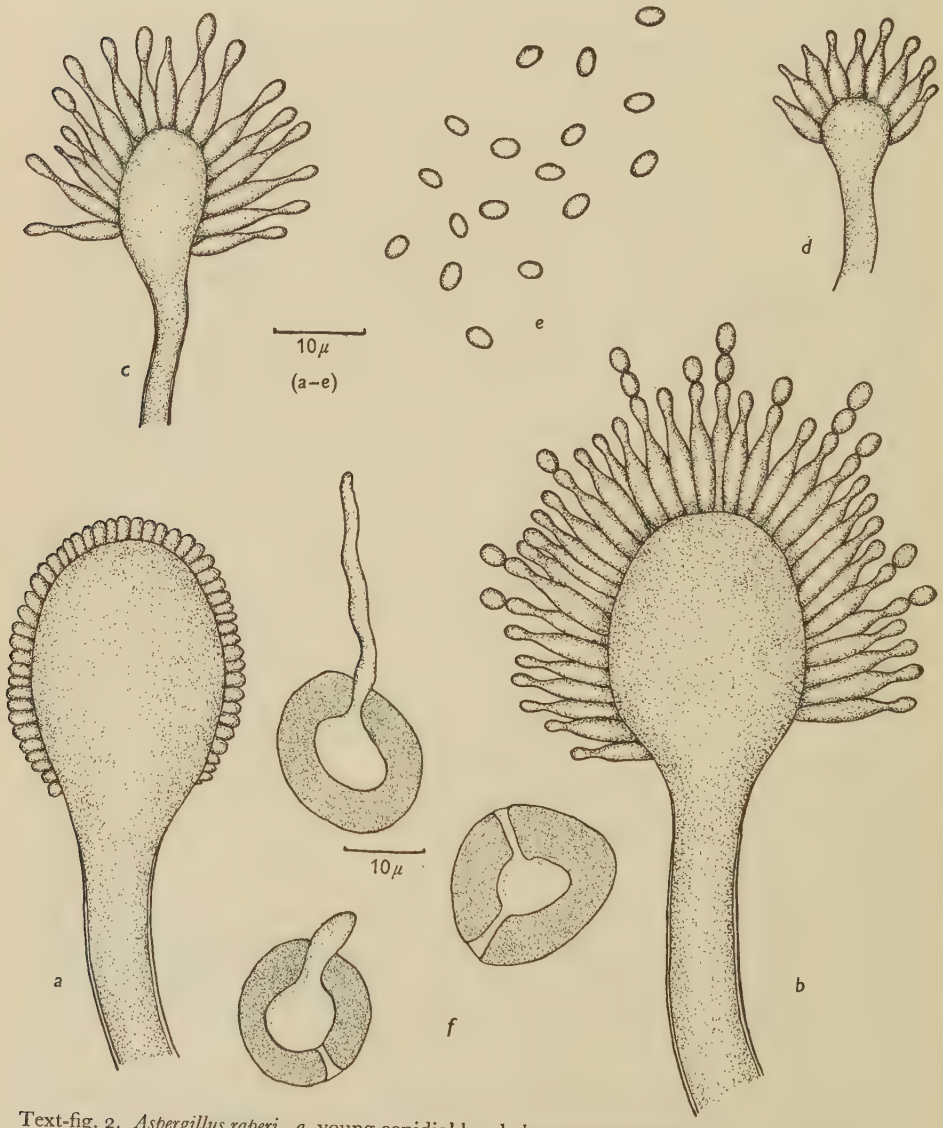
On account of its strongly coremiform aspect and its irregular, asymmetric penicilli, the fungus must be placed in the *P. claviforme* series (Raper & Thom, 1949). Within this series it is nearest to *P. clavigerum* Demelius, since this species is characterized by sterigmata of the same general character. Our fungus is distinguished from *P. clavigerum* by the much larger coremia, by the conspicuous yellow colour of the mycelium and by the somewhat pointed, spinulose conidia.

The fungus is named *P. isariaeforme* because of its cultural aspect, which strongly resembles that of the genus *Isaria*. We are indebted to Mr George Smith (London School of Hygiene and Tropical Medicine) for suggesting the specific epithet.

The type culture Pe 8 was isolated from woodland near Yangambi, Belgian Congo, in September 1949. Two additional strains were also isolated from woodland near Yangambi in December 1955. These strains agree in all characteristics with the type.

Aspergillus raperi sp.nov. (Pl. 8, figs. 4, 5; Text-fig. 2)

Coloniae, in agaro Czapeki bene crescentes, consistunt ex plano strato corpusculorum globosorum colore cremoris vel pallide flavo-brunneo ex cellulis 'hülle' dictis constantium, inter quae capitula cano-viridia, plerumque pauca numero; coloniae interdum marginem versus colore cano-viridi formatione conidiorum; margo coloniae irregulariter incisus; reversum flavum usque viridi-nigrum; capitula conidica plerumque pauca numero, interdum maiore numero, radiata, 200–300 μ in diam.; conidiophora oriuntur ut rami laterales mycelii aërii, usque 1000 μ longa, 6–12 μ lata, parietibus hyalinis glabris



Text-fig. 2. *Aspergillus raperi*. *a*, young conidial head; *b*, mature conidial head, showing only one series of sterigmata; *c–d*, reduced conidial heads; *e*, conidia; *f*, hülle cells.

1 μ crassis; vesiculae subglobosae, 14–40 μ in diam., per paene totam superficiem sterigmatibus tectae; sterigmata uno ordine, 7·5–9 × 2–2·5 μ; conidia hyalina, elliptica, glabra, 3–3·8 × 2–2·5 μ; corpuscula globosa pernumerosa, 1 mm. in diam., tota constantia ex cellulis 'hülle' dictis; cellulae 'hülle' dictae globosae usque subglobosae 24–36 × 22–29 μ. Hab. in solo Congo Belgicae, apud pagum Yangambi, Africa, leg. J. Meyer.

Colonies on Czapek agar in Petri dishes grow well, attaining a diameter of 4–6 cm. in 2 weeks at 25° C., they are plane, with central area somewhat raised and with colony margin strongly dissected. The fungus is especially characterized by the abundant production of hülle cells, mostly collected into definite bodies. These are produced over the whole colony area or in sectors, giving the colony a granular appearance (Pl. 8, fig. 4), in shades of Cream Color or Cream Buff, becoming Chamois in age; mostly azonate. Conidial heads are scattered among the hülle cells, in pale grey green shades, passing through Cream Color to Vetiver Green and Pea Green, in heavy-sporing strains giving the colonies—especially in outer areas—a definite grey green shade, near Sage Green. Exudate fairly abundant, in small pale yellow drops. Odour inconspicuous, somewhat mouldy, in some strains, however, fairly pronounced, resembling the odour of carbolic acid. Reverse of colonies mostly passing through shades of Wax Yellow and Primuline Yellow to Warm Buff and Old Gold with Clay Color beneath the hülle cells, and sometimes becoming Chaetura Black in central areas, surrounding agar usually in pale yellow shades near Cream Color.

Conidial heads (Text-fig. 2*a–b*) limited to fairly abundant, depending on the strain, radiate in pattern, sometimes approaching loosely columnar, 200–300 μ in diameter, borne on conidiophores originating from aerial hyphae or in marginal areas of older cultures also from submerged mycelium. *Conidiophores* mostly very long, up to 1000 μ in length, 6–12 μ in diameter with walls colourless and smooth, 1 μ thick. *Vesicles* subglobose, mostly 14–40 μ in diameter, bearing sterigmata over almost their entire surface. *Sterigmata* in one series, 7·5–9 × 2–2·5 μ. *Conidia* hyaline, elliptical, smooth, mostly 3–3·8 × 2–2·5 μ (Text-fig. 2*e*). *Hülle cells* abundant, globose to subglobose, 24–36 × 22–29 μ (Text-fig. 2*f*), collected into clusters, which are mostly surrounded by very thin envelopes of interlaced, pale yellow hyphae, producing globose bodies, about 1 mm. in diameter.

Colonies on malt agar grow somewhat more rapidly than on Czapek agar, consisting of a continuous layer of Cream Buff to Colonial Buff globose bodies; conidial heads limited in number, Vetiver Green to Pea Green, mostly not affecting the colony appearance; reverse in orange to brown shades. Structural elements of conidial heads as on Czapek agar.

The type strain 23717 was isolated from soil of grassland near Yangambi, Belgian Congo, in September 1953. The species is also represented by the strains 266 and 310, both isolated from the same source and differing only from the type in some minor characters, such as the less rapid growth of the cultures (Pl. 8, fig. 5) and the very limited number of the conidial heads. Strain 310 is especially characterized by the pronounced odour resembling that of carbolic acid. The three cultures in question have a conspicuous tendency to produce floccose, white sectors forming only a few conidial heads and no hülle cells. In consequence cultures in tubes may in a relatively short time degenerate into flocculent and only lightly sporulating

forms without the typical clusters of hülle cells, thus altering completely the cultural aspect.

In all three strains examined reduced conidial heads were observed (Text-fig. 2*c–d*). Additional strains were isolated from woodland near Yangambi.

The fungus cannot be placed in the present scheme of the genus *Aspergillus* as suggested by Thom & Raper (1945). According to Prof. Raper (*in litt.*) the best placement is in the *A. versicolor* group, since the radiate conidial heads, the subglobose vesiculae fertile over nearly their entire surface and the pattern of the hülle cells are characteristic for this group. This means, however, a broadening of the concept of the *A. versicolor* group to include a form with only a single series of sterigmata. The occurrence of one or two series of sterigmata does not always seem to be a suitable group characteristic, since also the *A. niger*, *A. tamarii* and *A. flavus-oryzae* groups contain species with double sterigmata as well as species with single sterigmata. The species is named in honour of Prof. Dr Kenneth B. Raper, to whom we are greatly indebted for many valuable suggestions.

REFERENCES

Raper, K. B. & Thom, C. (1949). *A manual of the Penicillia.* Baltimore.
Ridgway, R. (1912). *Color standards and color nomenclature.* Washington, D.C.
Thom, C. & Raper, K. B. (1945). *A manual of the Aspergilli.* Baltimore.

EXPLANATION OF PLATE 8

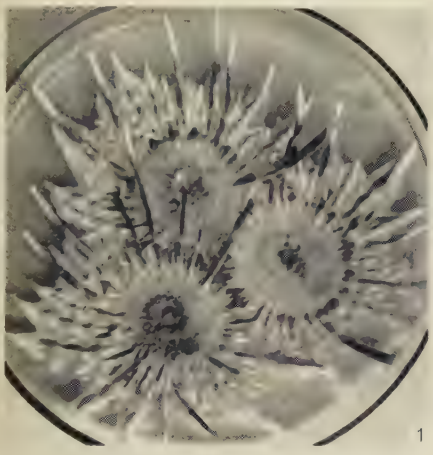
Figs. 1–3. *Penicillium isariaeforme*

Fig. 1. Star-like colonies of the type strain (Pe 8) on Czapek agar in a Petri dish.
Fig. 2. Young colony on Czapek agar in an Erlenmeyer flask, showing developing coremia.
Fig. 3. Coremia of a 1-month-old culture on malt agar in an Erlenmeyer flask, showing the typical alternating zones and the conspicuous white tips. The coremia are strongly positively phototropic.

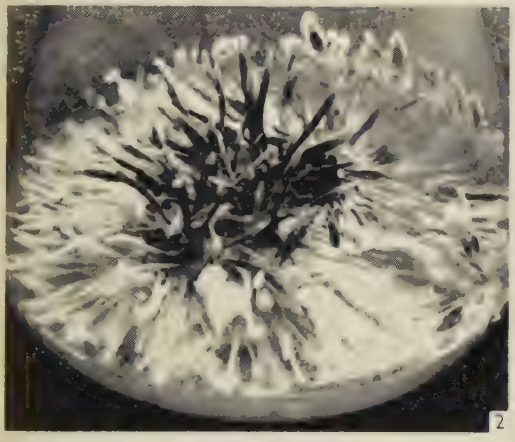
Figs. 4, 5. *Aspergillus raperi*

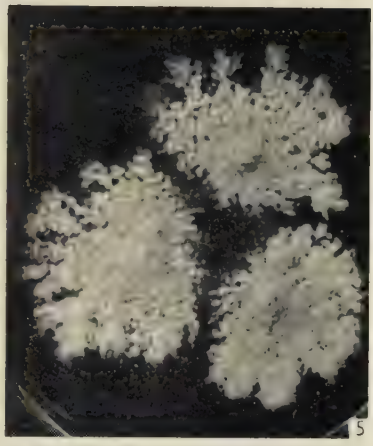
Fig. 4. Type strain 23717 on malt agar in a Petri dish at 2 weeks, showing masses of hülle cells collected into bodies, superficially suggesting perithecia.
Fig. 5. Strain 310 on malt agar in a Petri dish at 2 weeks.

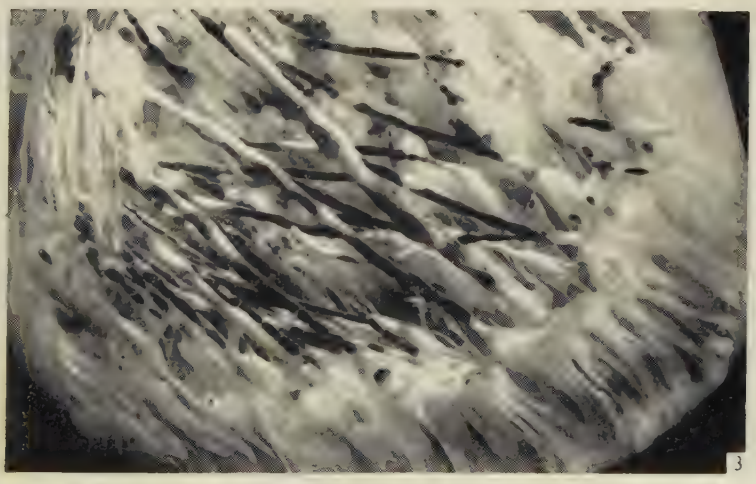
(*Accepted for publication* 28 *February* 1956)


1

4


2


5


3

(*Facing p.* 192)

Trans. Brit. mycol. Soc. **40** (2), 193–199 (1957).

NEW OR INTERESTING RECORDS OF BRITISH USTILAGINALES

By DEREK A. REID

Royal Botanic Gardens, Kew

(With 8 Text-figures)

Descriptions are given of a number of smuts, four of which are new to the British Isles—*Ustilago intermedia* Schroet., *Cintractia carpophila* (Schum.) Liro var. *carpophila*, *Entyloma ossifragi* Rostrup and *E. linariae* Schroet. Attention is drawn to the existence of a small-spored form of *Ustilago bullata* Berk. on *Bromus sterilis* and to new British hosts for a number of other species.

Ustilago bullata Berk., in Hooker, *Flora of New Zealand*, **2**, 196, 1855 (Fig. 1)

Syn. *Ustilago bromivora* (Tul.) Fischer von Waldheim in *Bull. Soc. Nat. Moscou*, **40**, 252, 1867.

To the list of British host plants for this smut given by Ainsworth & Sampson (1950) can be added *Bromus sterilis* and the cultivated grass, *B. briziformis*. Details of the latter collection are: *Habitat* on *B. briziformis*, Crouch End, 1909. No. A14949.

The occurrence of the fungus on *B. sterilis* is of special interest, as Liro (1924) stated that there was a small-spored form of *U. bullata* on *B. sterilis*, *B. tectorum* and *B. uniloides*. This collection is therefore described.

Sori in the spikelets replacing the floral parts. *Spore mass* hard, black, covered at first by host tissue. *Spores* globose-subglobose, yellowish brown, very minutely verrucose, 6–7 μ. *Habitat* on *B. sterilis*, Herbarium grounds, Royal Botanic Gardens, Kew, Surrey, coll. J. K. O'Byrne, 16 June 1955.

Ainsworth & Sampson (1950) give the spore size of *U. bullata* as 8–12 μ (mostly 9–10 μ). To confirm Liro's observation all the collections in the Kew Herbarium on *B. sterilis* were examined, and are listed below with their spore sizes. In all cases the exsiccatae were distributed under the name *U. bromivora*.

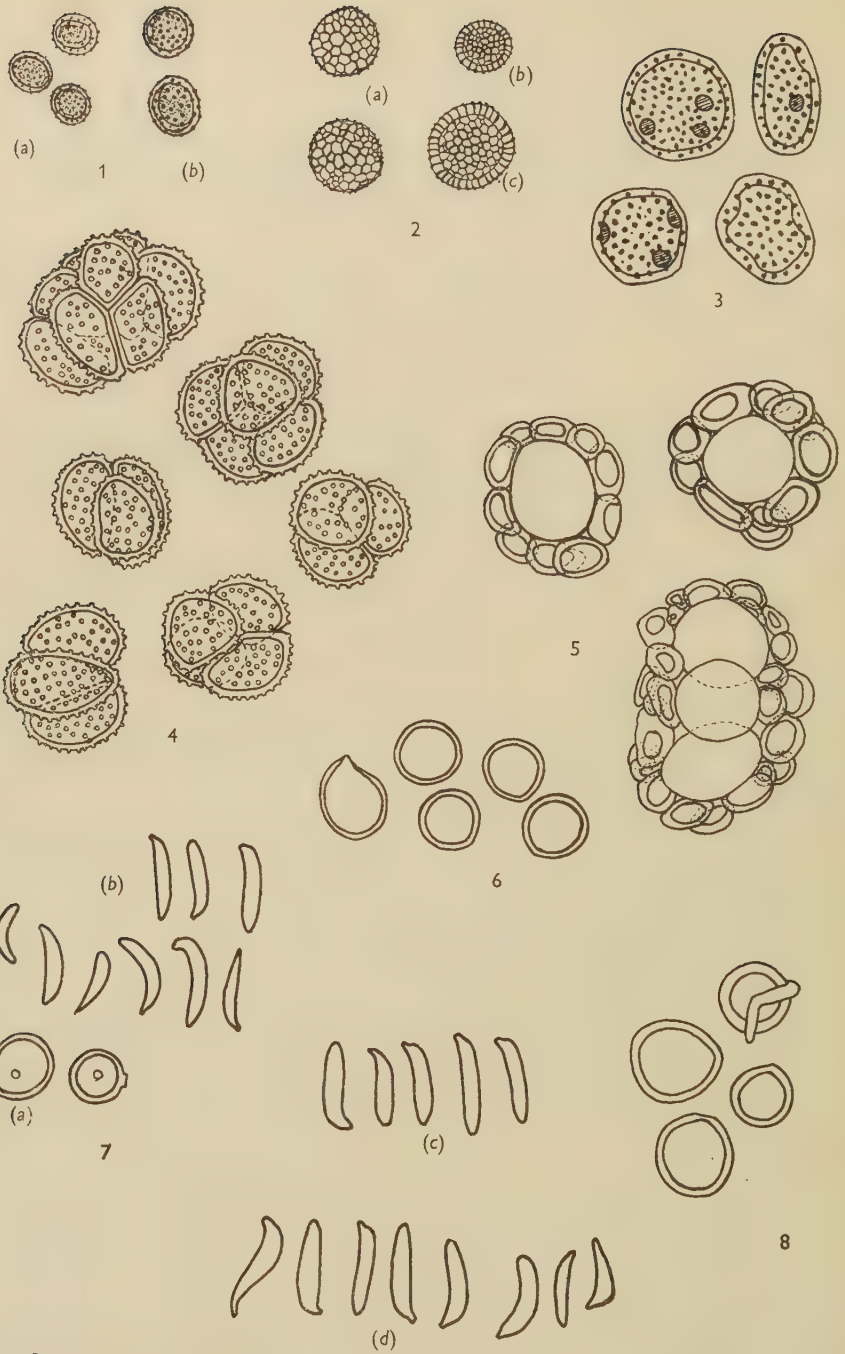
[1] J. Bornmüller: Lydiae et Cariae pl. exsiccatae, No. 10242, Lydia, Smyrna, 1906, V. Leg. et Det. J. Bornmüller et P. Magnus. *Spores* 6–8 μ.

[2] Bubák, Kryptogamae exsiccatae, No. 1901, Hungaria, Gellérthegy prope Budapest, M. Jun. Det. F. Bubák, Leg. J. Tuzson. *Spores* 5–8·5 μ.

[3] Flora Hungarica exsiccata, Cent. V, Fungi 41, No. 401, Budapest, territ. Farkasrét. Mens. Mai, 1916. Leg. Z. Zsák, Det. G. de Moesz. *Spores* 6–8 μ.

[4] Pollacci, Fungi Longobardiae exsiccatae, Papiam, Leg. M. Turconi. *Spores* 6–9 μ. This collection was found to be on *B. diandrus* (*B. gussonii*) and not *B. sterilis* as stated (vide C. Hubbard).

The spore measurements of the British collection and the first three



Figs. 1–8. Fig. 1. *Ustilago bullata*: (*a*) spores from *Bromus sterilis*; (*b*) spores from *B. uniloides*. Fig. 2. (*a*) *Ustilago intermedia*: spores; (*b*) *U. scabiosae*: spore; (*c*) *U. flosculorum*: spore. Fig. 3. *Cintractia carpophila* var. *carpophila*: spores. Fig. 4. *Thecaphora trailii*: spore-balls. Fig. 5. *Urocystis fischeri*: spore-balls (sterile cells overlying the spores not drawn). Fig. 6. *Entyloma holci*: spores. Fig. 7. *Entyloma helosciadii*: (*a*) spores, and (*b*) sporidia on *Oenanthe crocata*; (*c*) sporidia on *Apium nodiflorum*, Weaver bank collection; (*d*) sporidia on *A. nodiflorum*, Clondalkin, Co. Dublin, I.M.I. No. 32305. Fig. 8. *Entyloma ossifragi*: spores. All ×866.

gatherings cited above are uniformly small (5–8·5 μ) and support Liro's view of the existence of a small-spored race of the species on this host. In the course of this investigation British specimens of *U. bullata* on *B. uniloides* were found to have spores 8–10 μ which is within the usual range of this species. The spores of specimens on *B. mollis* were more variable ranging from 6 to 12 μ.

Ustilago hypodytes (Schlecht.) Fr., in *Syst. Mycol.* **3**, 518, 1832

Ainsworth & Sampson (1950) list *Bromus carinatus* as a British host of this smut. The record is, however, based on a mis-determined host which is in fact *B. erectus* var. *villosus*.

Ustilago intermedia Schroet., in *Rabenhorst Fung. Eur.* No. 1696, 1872 (Fig. 2)

Sori in the anthers. *Spore mass* powdery, very dark violet. *Spores* globose-subglobose, pale violet, reticulate, lacking a hyaline epispore, 9–15·6 μ (mostly 10–13 μ). The reticulation is remarkable for the very uneven width of the mesh which ranges from 1 to 3 μ in diameter on the same spore. *Habitat* on *Scabiosa columbaria*, Ivinghoe Hills, Bucks, coll. D. A. Reid, 3 August 1952; Eaton, Norfolk, coll. E. A. Ellis, 23 August 1955.

This species would seem to be confined to *S. columbaria* and Schröter (loc. cit.) observed that when *Knautia arvensis* grew amongst diseased plants of *S. columbaria* it remained free from infection. *U. intermedia* is easily separable from the anther smuts occurring on *Knautia* (*U. scabiosae* (Sow.) Wint. and *U. flosculorum* (DC.) Fr.) and *Succisa* (*U. succisae* Magn.) by the dark violet colour of its spore mass, and microscopically in having spores which lack a distinct hyaline epispore but which are covered by a reticulate ornamentation, the mesh of which is of very variable diameter.

U. intermedia was first reported from Britain by Cooke (1875) on flowers of *Scabiosa*, Scotland, coll. Rev. J. Fergusson, and this record was listed by Stevenson (1879). This collection is preserved in the Kew Herbarium and is *U. flosculorum* on *Knautia arvensis*. Later, Cooke (1902) recorded *U. intermedia* on florets of *Scabiosa columbaria*, but without specific reference to its presence in Britain. Plowright (1889) regarded *U. intermedia* and *U. succisae* as synonyms of *U. flosculorum* and gave *S. columbaria*, *S.* (*Knautia*) *arvensis*, and *S.* (*Succisa*) *succisa* as hosts of this species. It is not clear, however, if he had seen infected British material of *S. columbaria*, or whether he included it knowing that this plant was often infected with an anther smut in Europe. Confusion over synonymy probably led Zundel (1953) to state that *U. scabiosae* occurred on *S. columbaria* in Britain, and his record of *U. intermedia* on this host is probably based on Cooke's observations cited above. It would thus seem that previous reports of this smut in Britain were based on insufficient evidence, and that the author's collection in 1952 constitutes the first authentic record from this country.

Cintractia carpophila (Schum.) Liro, in *Die Ustilagineen Finnlands*, **2**, 27, 1938, var. *carpophila* (Fig. 3)

Sori replacing the ovaries, partly covered by the glumes, but very conspicuous, globose to subglobose 1·5–3 mm. in diameter. *Spore mass*

agglutinated, black, surrounding a central columella of host tissue. *Spores* in surface view rounded or slightly angular, but in side view elliptical, dark brown, with two to four internal swellings of the spore wall which may be either smooth or very minutely roughened when examined under an oil-immersion lens. There is, however, a faint granular ornamentation on the spore surface. *Habitat* on *Carex echinata*, near the summit of Carn Dearg Mor, Aviemore, Inverness-shire, at about 2000 ft., coll. D. A. Reid, 5 September 1955; on *C. echinata*, on the lower slopes of Cairn Gorm Mountain near Aviemore, Inverness-shire, coll. D. A. Reid, 6 September 1955. This would seem to be the first published record of *Cintractia carpophila* from the British Isles, but it is probable that Plowright's (1889) record of *C. caricis* (Pers.) Magn. on *Carex stellulata* [*C. echinata*] refers to this species. The fact that *C. echinata*, although a common plant, is so rarely found in a smutted condition, indicates that the fungus is of very rare occurrence in this country.

The author wishes to thank Dr D. B. O. Savile, Department of Agriculture, Ottawa, Canada, for confirming the identity of these specimens. He comments (*in litt.*) on the spores of the Cairn Gorm collection as follows: 'the walls are slightly thicker and more appreciably verrucose than typical, but are still not rough enough for var. *verrucosa*'; and on the Carn Dearg Mor collection: 'the spores are a trifle large, but the wall thickness, markings and swellings are well within the normal range'.

Thecaphora trailii Cooke, in *Grevillea*, **11**, 155, 1883 (Fig. 4)

Syn.: *Thecaphora cirsii* Boud., in *Bull. Soc. mycol. Fr.* **3**, 149, 1887.

Sori in the inflorescences entirely replacing the florets. *Spore mass* powdery, purplish brown. *Spore-balls* irregularly globose or elongate, 20·8–31·2 × 15·6–23·4 μ, consisting of 2–9 spores. *Spores* rather angular due to mutual pressure, with very minute reticulate ornamentation appearing as warts on the free convex surfaces, pale yellow, 10–18 μ. *Habitat* on *Cirsium dissectum* (= *C. pratensis*), Ballyvaughan, Co. Clare, Eire, coll. D. A. Reid, 5 July 1952.

All previous British collections of this smut have been found on *Cirsium heterophyllum* at Braemar, Aberdeenshire, and Henderson (*in litt.*) informs me that it is still to be found in this locality where it has persisted since at least 1873. This extremely local distribution is difficult to explain for the host plant is widespread in Scotland and northern England. The discovery of a new locality in Eire, first reported by Reid (1953), is therefore of some interest especially as there is no record of *T. trailii* having been collected on *C. dissectum*.

Urocystis fischeri Körn., in *Hedwigia*, **16**, 34, 1879 (Fig. 5)

Syn.: *Tuburcinia fischeri* (Körn.) Liro, in *Ann. Univ. åbo.* (*turku.*), Ser. A, **1**, 29, 1922.

Sori in the leaves, forming very elongated pustules between the veins, remaining covered by host tissue for some time, which makes detection difficult. *Spore mass* powdery, black. *Spore-balls* globose or elongated, 23·4–46·8 × 15·6–31·2 μ, consisting of 1–4 spores completely surrounded by

thick-walled yellowish brown sterile cells 7–10(–13) μ in diameter. *Spores* globose to ovate, dark reddish brown, 13–16(–18·2) × 13–15·6 μ. *Habitat* on *Carex flacca*, Ballyvaughan, Co. Clare, Eire, coll. D. A. Reid, 5 July 1952.

Apart from the gathering described above, reported by Reid (1953), there have been three British collections of this fungus: on *Carex* sp., Forfarshire, Scotland, coll. William Gardiner [Herb. Berkeley]; on *C. flacca*, John O'Groats, July 1947, coll. Dr E. Gray (vide Wilson & Henderson, 1952); Coombe Hill, Wendover, Bucks, Herb. Path. Lab. 12 (I.M.I. No. 46391), 29 June 1947. [Ainsworth & Sampson (1950) erroneously gave the county as Dorset.]

Entyloma holci Liro, in *Die Ustilagineen Finnlands*, **2**, 97, 1938 (Fig. 6)

Syn.: *Entyloma crastophilum* Sacc. p.p., in *Michelia*, **1**, 540, 1879.

Sori abundant in the leaves as minute round or elongate, swollen, black, rust-like pustules, up to 650 μ long and 130 μ thick, occupying the whole of the central tissues of the host and bounded only by the upper and lower epidermis. *Spores* globose to subglobose, brown, 9–13 μ, with walls up to 1·5 μ thick. In sections through the sori the spores are seen to be in well-defined groups, within which the individual spores are rather angular owing to mutual pressure. *Sporidia* not seen. *Habitat* on *Holcus lanatus*, West Sandwich, Isle of Yell, Zetland, near sea-level, coll. R. W. G. Dennis, September 1952.

It would seem that there is a second European species of *Entyloma* occurring on *Holcus*, i.e. *E. sydowianum* Ciferri which is a synonym of *E. brefeldi* Krieger, according to Liro (1938). Ciferri (1928) based his description of *E. sydowianum* partly on Sydow's Mycotheca Germanica No. 66, but chiefly on material in the Saccardo Herbarium. Examination of Sydow's exsiccata in the Kew Herbarium failed to reveal the smut. However, the following collection on *Holcus mollis* agrees well with Ciferri's description and is certainly not *E. holci*: Rabenhorst–Pazschke, Fungi europaei et extraeuropaei No. 4202 (Fungi Saxon. No. 1104), Saxonia: Prope Königstein a. Elbe, Juni 1898, Leg. W. Krieger. This species differs from *E. holci* in forming scanty, ill-defined, elongated, yellow spots, 2 × 4–5 mm., and in having hyaline or very pale yellow spores, 10–14 μ in diameter, with thinner membranes, which do not appear to form distinct groups within the sori. *E. holci* was first reported from Britain by Dennis & Gray (1954).

Entyloma helosciadii Magn., in *Hedwigia*, **21**, 129, 1882 (Fig. 7)

Syn.: ?*Entyloma oenanthes* R. Maire, in *Bull. Soc. bot. Fr.* **48**, 208, 1901.

Sori in the leaves, in amphigenous, whitish, more or less circular lesions, 0·5–4 mm. in diameter. *Spores* hyaline, globose, 7–12 μ with membranes 1–1·5 μ thick. *Sporidia on the host* more or less sickle-shaped, hyaline, 10–14 × 2–3 μ, borne on both surfaces. These sporidia are produced on parts of the lesions where spores are scanty and may be the result of secondary infection. *Habitat* on *Oenanthe crocata*, Cloutsham Valley, near Porlock, Somerset, coll. D. A. Reid, 1 September 1954.

There have been two species of *Entyloma* described on *Oenanthe* spp., viz. *E. oenanthes* R. Maire on *O. apiifolia* having spores 8–14 μ in diameter, and *E. debonianum* Sacc. on *O. globulosa* with spores 16–17 μ in diameter. The former is, however, almost certainly a synonym of *E. helosciadii*, but unfortunately there has been considerable confusion in the literature regarding its spore size. Saccardo (1905), owing to a printer's error, gave the spore dimensions as 8–4 μ, and Ciferri (1928, 1938) reversed these measurements citing them as 4–8 μ. There are no differences between the spores of the fungus on *Apium* and *O. crocata* and the conidia are also very similar, although often slightly narrower on the latter host. Ainsworth & Sampson (1950) gave the conidial size of the smut on *Apium* as 9–14 × about 3 μ, whilst I find it to be 10–18·2 × 3 μ. These observations, together with the fact that both plants occur in the same ecological habitat and belong to the same family, suggest that the fungi are conspecific.

To the records cited by Ainsworth & Sampson (1950) under *E. helosciadii* can be added: on *Apium nodiflorum*, Weaver Bank, Cheshire, coll. J. W. Ellis.

Entyloma ossifragi Rostrup, in *Ustilagineae Daniae*, 119, 1890 (Fig. 8)

Sori in the leaves as blackish, tar-like, circular or elongated lesions up to 1 cm. in length. These lesions often become confluent and may occupy the whole width of the leaves. *Spores* globose, yellowish brown, 9–15·6 μ, with membranes up to 2 μ thick. *Sporidia* not seen. *Habitat* on *Narthecium ossifragum*, base of Cnoc nan Cro, south of Laxford River, west coast of Sutherland, *c.* 150 ft., coll. R. W. G. Dennis, 21 September 1954. This is the first collection of the fungus since it was described 66 years ago.

REFERENCES

Ainsworth, G. C. & Sampson, K. (1950). *The British smut fungi.* (*Ustilaginales.*) Kew.

Ciferri, R. (1928). Quarta contribuzione allo studio degli Ustilaginales. *Ann. mycol., Berl.*, **26**, 1–68.

Ciferri, R. (1938). Ustilaginales. *Flora Italica Cryptogama*, **1**, fasc. 17.

Cooke, M. C. (1875). British fungi. *Grevillea*, **4**, 66–69.

Cooke, M. C. (1902). *Microscopic fungi*, 4th ed. London.

Dennis, R. W. G. & Gray, E. G. (1954). A first list of the fungi of Zetland (Shetland). *Trans bot. Soc. Edinb.* **36**, 215–223.

Liro, J. I. (1924). *Die Ustilagineen Finnlands.* **1**. Helsinki.

Liro, J. I. (1938). *Die Ustilagineen Finnlands.* **2**, Helsinki.

Plowright, C. B. (1889). *British Uredineae and Ustilagineae.* London.

Reid, D. A. (1953). Fungi from the Galway Bay area. *Irish Nat. J.* **11**, 64–5.

Saccardo, P. A. (1905). *Sylloge Fungorum.* **17**. Patavii.

Stevenson, J. (1879). *Mycologia Scotica.* Edinburgh.

Wilson, M. & Henderson, D. M. (1952). Some Scottish smut fungi. *Trans. bot. Soc. Edinb.* **36**, 72–3.

Zundel, G. L. (1953). *The Ustilaginales of the world.* Pennsylvania.

(*Accepted for publication* 29 *February* 1956)

ADDENDUM

Entyloma linariae Schroeter, in *Beiträge zur Biologie der Pflanzen* (*Cohn*), **2**, 371, 1877

Sori in the leaves, forming amphigenous or more frequently hypophyllous, pale yellowish, ovate lesions, up to 3·5 mm. in length. *Spores* golden-brown, globose to subglobose 11–15·6 μ, with conspicuous double walls, 1·5–2·5 μ thick, the outermost appearing almost hyaline. *Sporidia on the host* sickle-shaped, hyaline, 9–11 × 2–3 μ. *Habitat* on *Linaria vulgaris*, Wembury, South Devon, coll. E. A. Ellis, 28 October 1956.

Zundel (1953) stated that conidia (sporidia) were apparently lacking in *E. linariae*, but gave the size of the sporidia of *E. veronicae* (Wint.) Lagerh. as 27–30 × 2 μ. Fischer (*Manual of the North American smut fungi*, 1953) regarded *E. veronicae* as a synonym of *E. linariae* and stated that conidia of the latter species were 'sparingly produced, filiform, aciculate, to fusiform, frequently with bent or curved ends 1·5–2·5 × 20–35 μ', but gave no details as to the host plants from which these measurements were obtained. The fact that Zundel's measurements of the conidia of the *Entyloma* on *Veronica* spp. are so similar to those of Fischer for *E. linariae* suggests that the latter's measurements may also have been made from the fungus infecting *Veronica*. This view is further strengthened by the discovery of the present author of sporidia on the lesions of infected leaves of *Linaria vulgaris* which are very much smaller than those described by Fischer, and if correct provides some evidence for retaining *E. linariae* and *E. veronica* as distinct species.

I am greatly indebted to Mr E. A. Ellis for allowing me to publish this record.

Trans. Brit. mycol. Soc. **40** (2), 200–202 (1957).

THE TAXONOMY OF *STEREUM TRAPLIANUM* VELENOVSKÝ

By DEREK A. REID

Royal Botanic Gardens, Kew

(With 1 Text-figure)

A detailed description is given of the type specimen of *Stereum traplianum* Vel., and it is suggested that this species should be regarded as a synonym of *S. subpileatum* Berk. et Curt.

During a study of the stipitate species of the genus *Stereum* it was found necessary to examine type material of *S. traplianum* Vel., a portion of which is preserved in the Kew Herbarium. That this fungus was of special interest and had no relationship with any other stipitate species was clear from the original description and subsequent account by Pilát (1930). In this he stated that *S. traplianum* was unique and differed from all European members of the genus, but he had, unfortunately, overlooked certain important details of microstructure which would have enabled him to recognize its true affinities.

Stereum traplianum Vel., in *České Houby*, 759–760, fig. 136, no. 8, 1920 (Fig. 1)

Sporophore usually consisting of a number of thick (700–1200 μ), convex, flabellate to nearly spathulate, imbricate pilei, 3–6 cm. in diam., with lobate, flexuous margins. Each pileus is narrowed behind to form a short, black, lateral or almost lateral stipe, 0·5–1·0 cm. long, 0·6 cm. wide, which is smooth and glabrous. Upper surface, from which smaller lobes arise, zoned, blackish brown to practically black, at first hirsute, becoming more or less glabrous. Hymenial surface bright cream to yellowish ochre in dried material, with scattered brown spots, probably resulting from injury. Texture tough and leathery, becoming more woody and brittle when dry. *Trama* dimitic, consisting of unbranched, closely septate skeletal hyphae, 5–8 μ in diam., which have strongly thickened walls ($-2{\cdot}5\mu$), and freely branched, thin-walled generative hyphae 2·5–3 μ in diam., lacking clamp connexions at the septa. There is a very distinct golden-brown zone, 60–120 μ in thickness, forming the surface of the pileus. This has the same structure as the rest of the trama, but the thick-walled hyphae become coloured. The hairs forming the hirsute covering of the cap originate from this region, although mostly weathered away in the Kew material. *Hymenium* becoming stratose, reaching 312–351 μ in thickness at a point 2·0 cm. from the margin. Pilát (1930) states that there is a thin layer of scattered, rounded crystals, 10–12 μ in diam., at the base of each stratum,

but this was not observed, possibly due to the material having been soaked in potassium hydroxide before sectioning. *Cystidia* abundant up to 60 × 7 μ, of variable shape, mostly cylindrical or clavate, but those in the current basidial layer frequently have a distinct apical papilla. They are thick-walled except towards their extremities, although the walls of those in the uppermost hymenial stratum are appreciably thinner than the lower ones

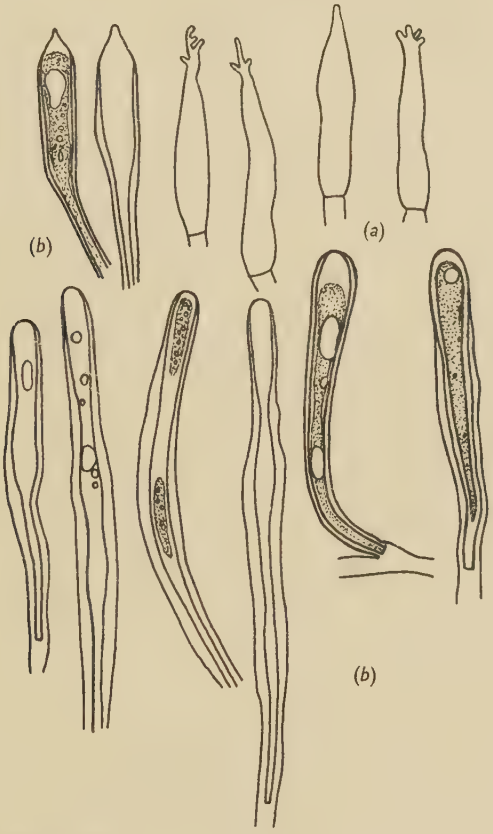


Fig. 1. *Stereum traplianum*: (*a*) paraphyses, × 1500; (*b*) cystidia, × 866.

which have become buried. These organs are devoid of encrusting material when examined in either potassium hydroxide or lactic acid. It is not clear how these structures in the lowermost hymenial stratum originate, as certain thick-walled hyphae curve into this zone from the trama, but whether they give rise to cystidia or terminate without modification is not known. However, those in the upper strata arise from thin-walled, branched generative hyphae, and it is probable that all the cystidia arise in this manner. Pilát (1930) said of the cystidia that they were not clearly differentiated, and that there were only a few nearly colourless somewhat thicker hyphae present. *Paraphyses* 18·2–26 × 3 μ, thin-walled, many with acute or obtuse spiny apices. *Basidia* not seen, but Pilát (1930) describes

them as being colourless, thin-walled, clavate, 30–40 × 3·5–5 μ, with two or four straight sterigmata 2–3·5 μ in length. *Spores* not seen.

Habitat. On beech-stumps in the Lower Tatra, at Báňská Bystrica, Czechoslovakia; coll. Prof. Trapl.

The microscopic structure of this fungus is very similar to that of *Stereum subpileatum* Berk. et Curt. In both, the cystidia are of similar appearance, and originate in the same manner, but whereas those of *S. subpileatum* are usually encrusted, those of *S. traplianum* are not. This difference is of no diagnostic value, as similar variation within species of the Thelephoraceae and Hydnaceae having encrusted cystidia is often encountered. Pilát (1930) further states that the cystidia of *S. subpileatum* are very variable according to the age of the fungus and the substratum. Both species have similar aculeate paraphyses, stratified hymenium and hyphal structure. The difference in coloration of the upper surface which in *S. traplianum* is almost black and in *S. subpileatum* is cinnamon brown, is almost certainly the result of weathering to which the type specimen of the former has undoubtedly been exposed. All other species of the genus with aculeate paraphyses are effuso-reflexed, and it seems very probable that the so-called stipe of *S. traplianum* is due to abnormal growth. As the two species cannot be satisfactorily separated it is proposed to reduce *S. traplianum* to synonymy under *S. subpileatum.*

S. subpileatum is a predominately tropical fungus occurring on deciduous trees. It is apparently fairly common in the southern United States, and has been recorded from Cuba, Venezuela and Brazil. In Europe it is very rare, having been collected in Austria, Czechoslovakia, Hungary and Italy. There is also a record from Siberia. In addition, there are collections in the Kew Herbarium from: (1) Cape Spartel, Tangiers, North Africa, coll. E. J. H. Corner, 15 April 1926; (2) Kasewe Hills (Kori), Sierra Leone, on dead parts of a living trunk of *Hormalium alnifolium*, coll. F. C. Deighton, (M4739) 16 June 1952.

REFERENCE

Pilát, A. (1930). Monographie der europäischen Stereaceen. *Hedwigia*, **70**, 10–133.

(*Accepted for publication* 25 *April* 1956)

Trans. Brit. mycol. Soc. **40** (2), 203–210 (1957).

STUDIES ON BRITISH SPECIES OF *ENDOGONE*. III. GERMINATION OF SPORES

By ROSEMARY M. GODFREY

Department of Botany, University of Bristol

(With 1 Text-figure)

Previous attempts to germinate spores of species of *Endogone* are described and discussed.

Attempts to obtain cultures of *E. lactiflua* from pieces of fruit body or from zygospores were unsuccessful. Zygospores were treated in a variety of ways, but none germinated. Limited experiments with *E. pisiformis* were also unsuccessful.

Chlamydospores of *E. microcarpa* and of a strain of *E. macrocarpa* germinated sporadically. External treatments did not influence the incidence of germination, but the age and condition of the spores was important.

Germination of chlamydospores invariably took place by the emergence of a germ tube through the stump of the parent hypha. A considerable development of a characteristically branched mycelium, often bearing small chlamydospores, followed germination, but attempts to grow this mycelium in pure culture failed. Hyphae ceased to grow when severed from the original chlamydospore or in the absence of contaminating organisms.

The type of germination provides evidence for the chlamydosporic nature of these spores, and makes it unlikely that they are parthenogenetic zygospores (azygospores).

Introduction

Cultural studies are essential to the elucidation of the taxonomic position of the genus *Endogone*. While most systematists are agreed that this genus is best considered as a highly specialized member of the Zygomycetes which shows some resemblances to the Hemi-Ascomycetes, definite proof of the affinities of *Endogone* might be obtained if the mode of germination of the zygospores were known. The suggestion that the chlamydospores of *Endogone* are in fact parthenogenetic zygospores, or azygospores (Bucholtz, 1912; Atkinson, 1918; *et al.*), is largely discounted by a study of the structure of these spores and of the dissimilarity between these and true zygospores where both occur in the same fruit-body (Godfrey, 1957*a*). Germination studies (Godfrey, 1955) provide further evidence of the chlamydosporic nature of these spores.

Kanouse (1936) succeeded in growing *E. sphagnophila* and *E. occidentalis* in pure culture from small portions of fruit-body. This work showed conclusively that the sporangial fruit-bodies previously attributed to *Endogone* were, in fact, not closely related to that genus, and resulted in their transfer to the new genus *Modicella*.

Attempts were therefore made to culture the British species of *Endogone* from pieces of fruit-body or from spores, by a variety of methods. Only a few experiments were successful, but these gave valuable information relating to the nature of the chlamydospores.

Methods

The fruit-bodies were heavily contaminated with other soil organisms, particularly when, as in those of *E. macrocarpa*, finely divided humus was present inside the gleba. Surface sterilization was useless, and when efficient killing of the contaminants was obtained by soaking the pieces of fruit-body in 1·0 % mercuric chloride, or dilute solutions of sodium hypochlorite or Chloramine T, the hyphae and probably also the spores of the *Endogone* were almost certainly injured. Repeated washing of pieces of fruit-body in sterile water and the addition of aureomycin to the isolation media considerably reduced the amount of bacterial contamination. Either the washed pieces of fruit-body or spores teased out from these were plated out on various media.

Attempts to culture *Endogone lactiflua*

Kanouse (1936) obtained cultures of *E. sphagnophila* and *E. occidentalis* by placing pieces of fruit-body on malt agar at 12° C. This simple method was not successful with *E. lactiflua* in the present study, nor did altering the temperature of incubation or substituting different nutrient agar media induce growth of the glebal hyphae or germination of the zygospores. Accordingly, a number of special treatments of the spores, including methods generally used to stimulate germination and attempts to rupture the continuous endospore or to render it permeable by removal of the chitin by the action of chitinase, were tried, but without success. The exospore was removed by dissection in some experiments, and in a few instances the endospore was also removed, leaving the spore contents surrounded by a thin membrane. The latter disintegrated on contact with water and complete removal of the endospore was obviously too drastic a treatment.

Although none of the spore treatments induced germination of the zygospores they are listed as evidence of the resistant nature of these spores:

(i) *Agar media used with and without aureomycin.* Water agar (agar 15 g., water 1 l.), water agar + 0·5 % tartaric acid, water agar + 0·25, 0·5 or 1·0 % sodium acetate, water agar + an extract of larch roots, prune extract agar, potato extract agar, potato extract agar + 0·5 % tartaric acid, 3·0 % malt extract agar (pH adjusted to give range from 3 to 10), 1·0 % malt extract agar, water agar + extract of soil from wood, where *E. lactiflua* was commonly found, Czapek's agar, 10 % glucose agar. A preliminary washing of the spores in bile salts was given in some instances since those are known to induce germination of spores of certain Myxomycetes (Elliott, 1949).

(ii) *Substrates other than agar.* Filter-paper soaked in distilled water, 0·25, 0·5 or 1·0 % sodium acetate, 0·01, 0·1, 0·5, 1·0 % furfural (since this substance has been generally used to induce germination of ascospores of *Neurospora* spp.; Catcheside, 1951), 1·0 % malt extract, or yeast extract. Cultures were incubated at 20° C. in light or in darkness. After 1–4 weeks some of the spores were washed and transferred to agar media.

(iii) *Temperature of incubation.* Usually 20° C. but 12, 25 and 30° C. were also tried.

(iv) *Pre-treatment at constant temperatures.* Portions of fruit-body on plates of agar or in tubes containing sterile water were incubated at temperatures ranging from −16 to 50° C. for periods of from ½ hr. to 10 months and were then transferred to 20° C.

(v) *Pre-treatment with alternating temperatures.* Alternation of 24 hr. at −16° C. or −20° C. with 8 days at room temperature, 12 hr. at +5° C. with 7 days at room temperature, 2 hourly alternation of 5° C. and room temperature during the day followed by room temperature at night. Samples of spores were plated out at regular intervals (48 or 24 hr.) during this experiment.

(vi) *Alternate wetting and drying.* Portions of fruit-body air-dried during day, soaked in sterile water during night. Some spores plated out after each 24 hr. period. Experiment carried on for 2 weeks.

(vii) *Mechanical injury of spore wall.* Portions of fruit-body ground up with powdered glass or carborundum powder; shaken in water with rubber-covered glass rods for ½, 2, 22, or 33 hr. according to the method devised by Jarman (1954) for the separation of plant tissues; spores compressed between sheets of glass.

(viii) *Treatment with hydrochloric acid.* 11N for 1–30 min., 2N, N, N/5, N/20, N/50, N/100, N/200 for 18 or 168 hr.

(ix) *Treatment with enzymes.* Spores fed to snail (*Helix pomatia* which has a chitinase enzyme in its gut; Karrer & Hoffmann, 1929), mouse, rabbit and chick, recovered from faeces, washed and incubated on agar; spores subjected in various ways to the action of living *Badhamia utriculosa* (which is able to attack the fungus *Stereum hirsutum* and thus probably secretes chitinase), *Aspergillus oryzae*, or various chitinase-producing bacteria and Actinomycetes isolated from woodland soil where *Endogone lactiflua* was found, or from garden soil, by the method described by Reynolds (1954), and to extracts of *Aspergillus niger* (said to be effective in degrading chitin, Grassman & Rubenbauer, 1931); spores soaked in extracts of mouse or rabbit dung for various periods (dung infusion was shown by Cheo & Leach (1950) to stimulate the germination of spores of *Ustilago striiformis*).

(x) *Presence of living tree roots.* Spores were plated out on agar in which spruce seedlings were growing under aseptic conditions, or placed in contact with an artificial 'rhizosphere' consisting of a sausage-shaped dialysing membrane filled with wet soil and larch roots, part of which was coated with plain agar and part was allowed to dip into water.

(xi) *Association with* Rhodotorula *spp.* Portions of fruit-body and spores placed on agar adjacent to colonies of *Rhodotorula* spp. found by Fries (1943) to stimulate germination of spores of certain Hymenomycetes.

(xii) *Storage.* Mature fruit-bodies crushed on a glass slide, covered with moist soil and stored at room temperature for 5 months before being plated out; whole fruit-bodies stored in sterile or non-sterile soil in cold greenhouse for periods of 2–17 months at various times of the year, then plated out; spores from air-dried fruit-bodies plated out after 1–5 years' storage.

None of these treatments induced growth of hyphae or germination of zygospores of *Endogone lactiflua.* Since only one fruit-body of the zygosporic

E. pisiformis was available exhaustive experiments could not be attempted with this species, but zygospores and portions of fruit-body were plated out immediately and after 9 months' storage at 6° C. without success. The resistant nature of zygospores of the Mucorales has been noted by many workers, and there are few accounts of the successful germination of even the commoner species. The zygospores of *Endogone* are obviously highly resistant to the effect of external stimuli.

Attempts to culture chlamydosporic species

Spores and pieces of fruit-body were treated similarly to those of *E. lactiflua*. Germination of the chlamydospores of *E. microcarpa* and the golden-spored variety of *E. macrocarpa* took place sporadically, but was not noticeably influenced by any of the special treatments used. The hyphae of the gleba never grew out from pieces of fruit-body. Germination was determined by the age and condition of the fruit-bodies at the time of collection. The wide occurrence of parasitism (Godfrey, 1957*b*) was no doubt largely responsible for the sporadic germination of spores of *E. microcarpa* and for the failure to germinate of spores of the yellow-spored variety of *E. macrocarpa*, of *E. fuegiana* and a single fruit-body of the *E. fasciculata–E. vesiculifera* group. It does not explain the failure to germinate of spores of the brown-spored and orange-spored varieties of *E. macrocarpa* which were never parasitized.

Germination of chlamydospores of *E. microcarpa* was obtained most frequently with young fruit-bodies in which the spores had reached maximum size, but which were still relatively thin-walled. Fruit-bodies at this stage were commonly collected in the autumn. Young spores from older fruit-bodies also germinated, while more mature ones from the same specimens did not do so. In the spring a number of very old fruit-bodies in which the spores were mature, were collected and about 20% of the spores in these germinated. Only those spores in which the entrance to the spore from the parent hypha was clear (Godfrey, 1957*b*) were able to germinate. Such a distribution of ability to germinate suggests that the spores are either physiologically mature before the wall reaches its maximum thickness or that they still retain their hyphal nature at this stage and are able to continue growth when detached from the parent hypha. As described below (p. 207) the stump of the parent hypha may itself resume growth. In view of the fact that germination invariably takes place through the stump of the parent hypha (Godfrey, 1955), and the observation that only those mature spores in which the connexion between this stump and the spore is open are able to germinate, it is unlikely that changes in wall structure influence renewed capacity for germination. One must suppose that the spore itself undergoes a period of dormancy before germination is once more possible. The experiments on pretreatment of spores have not so far succeeded in reducing this period of dormancy. Changes in endospore structure with age, however, might be necessary to allow the stimuli of external conditions to act on the spore contents in such a way as to end the dormancy. Material stored in damp filter-paper at 5° C. or in water at 25° C. or 5° C. for 3 months or in damp soil for 6–15 months provided germinatable spores indicating the resistant nature of the mature wall and

its success in protecting the contents from external influences for periods of several months.

In contrast to the effect of age on germination of chlamydospores of *E. microcarpa*, no immature thin-walled spores of *E. macrocarpa* were observed to germinate. Once the spores became mature, as judged by the thickness of the endospore a percentage germination of 70–90 % was obtained on water agar. This figure declined if the fruit-bodies were stored for any length of time. For example, chlamydospores from one fruit-body showed 70 % germination when plated out immediately after collection, and this dropped to 12 % after 3 months' storage in a cold greenhouse, whether or not a pre-treatment at 25° C. was given. It seems likely that the spore contents undergo a brief period of dormancy from the time of their formation until maturity or that maturity and ability to germinate are not attained until after maximum thickness of the wall is developed, but that the wall is less restrictive in its influence on the transmission of external stimuli than is that of *E. microcarpa*.

Mode of germination and growth

The mode of germination and the type of mycelium produced is similar in *E. microcarpa* and *E. macrocarpa*. Germination of *E. microcarpa* spores takes place on water agar at 20° C. after a minimum of 3 days, but sometimes it is delayed for a week or more. *E. macrocarpa* spores germinate within a week. Germination of the spores from any one fruit-body is spread over several days. With *E. microcarpa* it was noticeable that the spores from young fruit-bodies tended to germinate more quickly than those from old specimens.

When the chlamydospores are plated out, a stump of the parent hypha remains attached to the spore, and it is from this stump that the new hypha is produced. The germ tube can be seen passing along the parent hypha and emerging from the broken end. In young spores of *E. microcarpa*, a true germination does not always occur and, instead, growth is renewed from the end of the parent hypha. The growth form of the new hypha is precisely the same, but its wall is continuous with that of the parent hypha, instead of arising from the spore itself (Fig. 1).

A swelling is often observed at the point where the young hypha emerges from the old. In some cases the hypha grows forward and remains for a time unbranched, but, in others, several branches of approximately the same size are produced when the hypha emerges. The average rate of growth after germination is 2,550 μ in 20 days for *E. microcarpa*, and 1,960 μ for *E. macrocarpa*, but the rate of growth is extremely variable.

The hyphae are at first non-septate, but later become septate, particularly in the side branches. Hyphae in which septa are formed usually become empty of protoplasmic contents. Branching is infrequent. The hyphae grow mainly along the surface of the agar, but sometimes grow downwards into it and later return to the surface. The lateral branches nearly always grow out at right angles from a hypha, and this right-angled branching is a characteristic feature to be seen also in the fruit-bodies. The lateral branches may be branched also and the ultimate branchlets are fine and twisted. The principal hyphae of the mycelium in *E. microcarpa* are

about 4 μ wide with side branches tapering to 2 μ or less. In *E. macrocarpa*, the main hyphae are about 8 μ, but they may reach as much as 26 μ. The larger hyphae are thick-walled and yellowish in colour. Anastomosis occurs frequently between branches of the same hypha or between hyphae from different chlamydospores. The anastomoses are nearly always between hyphae which meet at right angles.

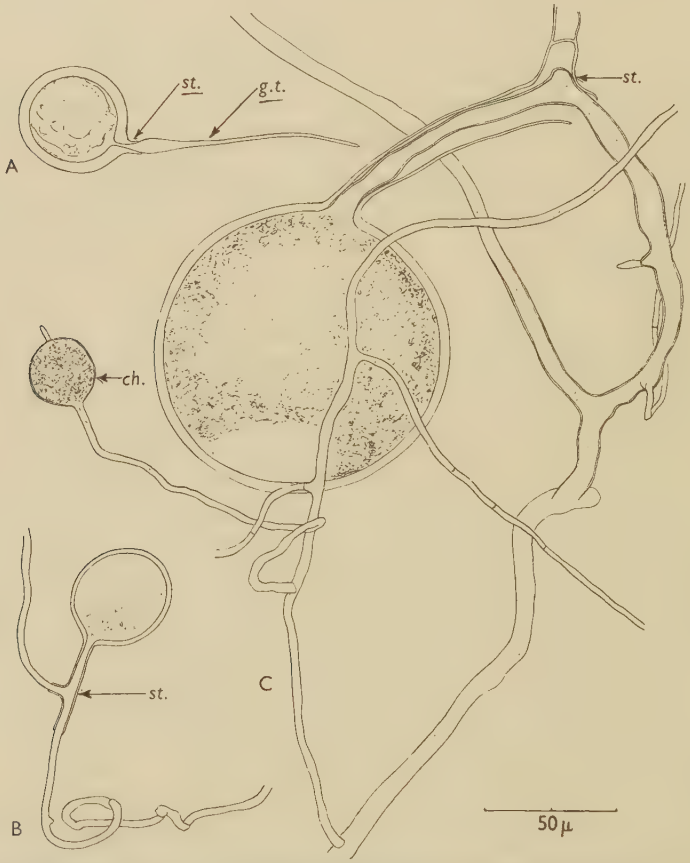


Fig. 1. *Endogone microcarpa*. A, germinating chlamydospore with germ tube (*g.t.*) emerging through stump of parent hypha (*st.*). B, later stage in which germ tube has branched, one branch emerging from a broken branch of original parent hypha. *Endogone macrocarpa*. C, extensive mycelium developing from germ tube which emerged through broken stump of parent hypha (*st.*). A young chlamydospore (*ch.*) is developing on this mycelium.

In some cultures small chlamydospores were formed on the mycelium. These were usually solitary and infrequent. Other cultures produced no chlamydospores at all, while in one culture of *E. macrocarpa* they were formed in great abundance. In *E. microcarpa*, chlamydospores were not formed when the cultures were derived from some of the more immature fruit-bodies. In *E. macrocarpa*, no definite correlation with age was noted, but there is need for further experimental data. The chlamydospores were

terminal or intercalary, and formed on the side branches of the mycelium. They remained comparatively thin-walled, the wall being not more than 1 μ in thickness. In *E. microcarpa* they measured about 20 μ in diameter, and in *E. macrocarpa* about 35 μ. At first the spores appeared to be empty (and often remained so in cultures of *E. microcarpa*) but on maturing they became filled with dense granular protoplasm.

Some contaminating fungi and bacteria were invariably present on the agar plates, despite all precautions in washing the spores. They rapidly spread over the plates, out-distancing the slow-growing *Endogone*. Attempts to obtain pure cultures met with no success. Hyphal tips, portions of hyphae, and germinating spores were transferred to fresh plates of agar, but they either failed to grow, or remained contaminated. When small pieces of agar bearing hyphae of *E. macrocarpa* still attached to chlamydospores were transferred to plain agar, renewed growth took place from the cut ends of the hyphae, but the culture could not be freed from contaminants. If the hyphae were not left attached to chlamydospores, no growth took place. Renewed growth from the inoculum never took place when *E. microcarpa* was similarly transferred.

It seems that the nutritional requirements of the hyphae are not satisfied on water agar unless the hyphae are still in communication with the food store in the spore. It is possible that they would grow on a richer medium, but every attempt has been unsuccessful because of an excessive growth of contaminants. The best solution to the problem seems to lie in effectively cleaning the spores before they are plated out. Not least of the difficulties is that of obtaining spores capable of germinating.

The germination of the spores of *E. microcarpa* and *E. macrocarpa* to give an extensive mycelium bearing secondary chlamydospores supports the view that the spores of these species are chlamydospores and not azygospores. If they had been parthenogenetic zygospores one would have expected that they would germinate to form a sporangium as in other Mucorales (Blakeslee, 1906). The mode of germination of the spores from the unidentified fruit-bodies described by Mosse (1954) resembled that of the chlamydospores of *E. microcarpa* and *E. macrocarpa*, and is evidence that these, too, are true chlamydospores, and not azygospores as has been claimed.

The 'chlamydospores' which Kanouse (1936) obtained in cultures grown from pieces of the fruit-body of the zygosporic *E. sphagnophila* did germinate to produce a sporangium of *Mucor* type. They possessed a continuous endospore, in contrast to the chlamydospores from the fruit-bodies of *E. microcarpa* and *E. macrocarpa* and also differed from these in staining reactions. These so-called 'chlamydospores' in Kanouse's cultures may have been azygospores.

The fact that chlamydospores germinated under conditions which did not induce germination of zygospores emphasizes the resistant nature of the latter.

Thanks are due to Dr L. E. Hawker for suggesting this problem and for assistance in the preparation of the manuscript. This paper and the two preceding ones of this series form part of a thesis accepted for the degree of Ph.D. in the University of Bristol.

REFERENCES

Atkinson, G. F. (1918). The genus *Endogone*. *Mem. Brooklyn bot. Gdn.* **1**, 1–17.

Blakeslee, A. F. (1906). Zygospore germinations in the Mucorineae. *Ann. mycol., Berl.*, **4**, 1–28.

Bucholtz, F. (1912). Beiträge zur Kenntnis der Gattung *Endogone* Link. *Beih. bot. Zbl.* **39**, 147–224.

Catcheside, D. C. (1951). *The genetics of microorganisms*. London.

Cheo, P. C. & Leach, J. G. (1950). The stimulating effect of dung infusion on the germinating spores of *Ustilago striiformis*. *Phytopathology*, **40**, 584–589. (Abstr. in *Rev. appl. Mycol.* **29**, 565.)

Elliott, E. W. (1949). The swarm cells of Myxomycetes. *Mycologia*, **41**, 141–170.

Fries, N. (1943). Untersuchungen über Sporenkeimung und Mycelientwicklung bodenbewohnender Hymenomycetes. *Symb. bot. Upsaliensis*, **6**, 1–81.

Godfrey, R. M. (1955). Germination of chlamydospores in the Endogonaceae. *Nature, Lond.*, **175**, 468.

Godfrey, R. M. (1957*a*). Studies on British species of *Endogone*. I. Taxonomy and morphology. *Trans. Brit. mycol. Soc.* **40**, 117–135.

Godfrey, R. M. (1957*b*). Studies on British species of *Endogone*. II. Fungal parasites. *Trans. Brit. mycol. Soc.* **40**, 136–144.

Grassman, W. & Rubenbauer, H. (1931). Über Zellulase und Hemizellulase mit besonderer Berücksichtigung ihrer therapeutischen Anwendung. *Münch. med. Nschr.* **18**, 1817.

Jarman, C. G. (1954). Mechanical separation of plant tissues. *Nature, Lond.*, **174**, 890.

Kanouse, B. B. (1936). Studies of two species of *Endogone* in culture. *Mycologia*, **28**, 47–62.

Karrer, P. & Hofmann, A. (1929). Polysaccharide XXXIX. Über den enzymatischen Abbau von Chitin und Chitosan. I. *Helv. chim. Acta*, **12**, p. 616.

Mosse, B. (1954). Studies on the endotrophic mycorrhiza of some fruit plants. Ph.D. Thesis, Univ. Lond.

Reynolds, D. M. (1954). Extracellular chitinase from a *Streptomyces* sp. *J. gen. Microbiol.* **11**, 150–159.

(*Accepted for publication* 9 *April* 1956)

Trans. Brit. mycol. Soc. **40** (2), 211–212 (1957).

HANSENIASPORA VINEAE SP.NOV.

By J. P. van der Walt and Irmgard T. Tscheuschner

National Chemical Research Laboratory, South African Council for Scientific and Industrial Research, Pretoria, South Africa

(With 1 Text-figure)

A new sporulating apiculate yeast isolated from vineyard soil in South Africa.

In the course of a survey of the yeast flora associated with surface soils in South Africa, a single strain of a hitherto undescribed *Hanseniaspora* species was isolated from vineyard soil.

The following description is based on the standard methods of Lodder & Kreger-van Rij (1952) for the identification of yeasts.

Growth in malt extract: after 3 days at 25° C. the cells are lemon-shaped, oval or long-oval (3·8–6·5) × (6·2–15·2–27) μ, single or in pairs. A thin sediment is formed. After 1 month a sediment, ring and few islets are formed. *Growth on malt agar*: after 3 days at 25° C. the cells are lemon-shaped, oval or long-oval (3·8–7·5) × (5·0–15–22) μ. After 1 month at 17° C., the streak culture is yellowish brown, almost smooth and somewhat shiny. *Slide cultures*: a primitive pseudomycelium is formed. *Sporulation*:* the spores are round, oval or slightly angular. They have a distinctly warty cell wall (Fig 1). No conjugation was observed.

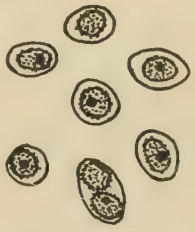


Fig. 1. *Hanseniaspora vineae* sp.nov. Ascospores on Gorodkowa agar after 7 days (× 1400).

Fermentation: glucose +, maltose −, galactose −, lactose −, saccharose −, *Sugar assimilation*: glucose +, maltose −, galactose −, lactose −, saccharose −. *Ethanol as sole source of carbon*: no growth. *Assimilation of potassium nitrate*: absent. *Splitting of arbutin*: absent.

Discussion

Although the existence of 1–2 round to oval ascospores in apiculate yeasts, as described by Niehaus (1932) and also by Dvornik (1938), was doubted by Sacchetti (1939) and by Henrici (1940), experience showed that there was no doubt as to the true ascosporogenous character of the endogenous bodies observed on sporulation media in the isolate studied by us. Not only were these bodies acid-resistant and acidophilic in their behaviour towards dyes, but they were also observed to germinate and were also more heat-resistant than the vegetative cells. These results are also in accordance with the observations of Shehata, Mrak & Phaff (1955).

* Spores were best observed on Gorodkowa agar.

Niehaus (1932) created the new genus *Kloeckerospora* for the classification of ascosporogenous apiculate yeasts forming 1–2 round ascospores. We prefer, however, to follow Mrak & Phaff (1948) and El Tabey Shehata *et al.* (1955), and include this species in the older genus *Hanseniaspora* Klöcker. It is, however, not possible to identify this species with any of the hitherto described sporogenous apiculate yeasts, fermenting and assimilating only glucose, viz. *H. valbyensis* Klöcker and *H. uvarum* (Niehaus) Shehata, Mrak & Phaff. Lodder & Kreger-van Rij (1952) give cell measurements in malt extract for the former species as $(2–4) \times (4–10)\,\mu$, while for the latter species Niehaus (1932) gives the cell measurements as $(3–4{\cdot}5) \times (6–8)\,\mu$ and $(3–3{\cdot}5) \times (12–15)\,\mu$. Comparison of the cell measurements of the strain from vineyard soil, measuring $(3{\cdot}8–6{\cdot}5) \times (6{\cdot}2–15{\cdot}2–27{\cdot}0)\,\mu$ in malt extract, makes it impossible to identify this strain with either *H. valbyensis* or *H. uvarum*. Furthermore, the strain from soil shows two additional minor morphological differences from the two species, namely the abundant formation of a primitive pseudomycelium and the marked wartiness of the ascospore wall. We therefore consider the strain under discussion as representative of a new species for which we propose the name:

Hanseniaspora vineae sp.nov. (Fig. 1)

In musto maltato cellulae ovidiae, longovidiae aut apiculatae $(3{\cdot}8–6{\cdot}5) \times (6{\cdot}2–15{\cdot}2–27{\cdot}0)\,\mu$ singulae aut binae. Sedimentum, insulae et anulus subtilis formantur.

In agaro maltato cellulae ovidiae, longovidiae aut apiculatae $(3{\cdot}8–7{\cdot}5) \times (5{\cdot}0–15–22)\,\mu$, singulae aut binae. Cultura (post unum mensem 17° C.) flavifusca, prope plana parum nitens. Pseudomycelium formatur. Ascosporae rotundae ovidiaeque; 1–2 in asco. Parvae verrucae in membrano ascosporae sunt.

Fermentatio glucosi solius. In medio minerali cum glucoso solo crescit. Nitras kalicus non assimilatur. In medio minerali cum alcohole aethylico non crescit. Arbutinum non finditur.

Subcultures have been deposited at the Centraal Bureau voor Schimmelcultures.

This paper is published by permission of the South African Council for Scientific and Industrial Research.

REFERENCES

Dvornik, R. (1938). Über die Sporulation der Apiculatushefen. *Zbl. Bakt.* (*Abt.* II), **98**, 315–344.

Henrici, A. T. (1940). The yeasts. *Bact. Rev.* **5**, 97–179.

Lodder, J. & Kreger-van Rij, N. J. W. (1952). *The yeasts*. Amsterdam: North Holland Publishing Co.

Mrak, E. M. & Phaff, H. J. (1948). Yeasts. *Ann. Rev. Microbiol.* **2**, 1–46.

Niehaus, C. J. G. (1932). Untersuchungen über Apiculatushefen. *Zbl. Bakt.* (*Abt.* II), **87**, 97–150.

Sacchetti, M. (1939). Sulla sporoficazione dei leiviti apiculati. *Ric. sci.* **10**, 422–426.

Shehata, A. M. El Tabey, Mrak, E. M. & Phaff, H. J. (1955). Yeasts isolated from *Drosophila* and from their suspected feeding places in Southern and Central California. *Mycologia*, **47**, 799–811.

(*Accepted for publication* 17 *April* 1956)

Trans. Brit. mycol. Soc. **40** (2), 213–220 (1957).

SPOROPHORE PRODUCTION BY *POLYPORUS ARCULARIUS* BATSCH EX FR. IN CULTURE

By I. A. S. GIBSON and JENNIFER TRAPNELL
Forest Department, Nairobi, Kenya

An isolate of *Polyporus arcularius* obtained from rotted pine was found to form complete sporophores in culture on malt agar. Sporophore formation in culture was dependent on a genetical factor. Fertile strains formed sporophores only in the light and on media containing growth factors of the vitamin B group. Blue light was most effective in stimulating sporophore formation and the number of sporophores increased in proportion to length of exposure. Acid media favoured sporophore formation and media containing a high level of nitrogen inhibited it. The latter was due, at least in part, to the accumulation of alkaline staling products.

Experimental studies on the fructification of members of the Agaricales and Polyporales in pure cultures have been few compared with those for other groups. Recently Hawker (1942) and Plunkett (1953) have made physiological studies, but other workers have concentrated on the growth of sporophores in culture for purposes of identification only (Long & Harsch, 1918; Etter, 1928; Badcock, 1943).

Polyporus arcularius Batsch. ex Fr. has a wide distribution as the cause of a white rot in fallen timber. Etter (1928) found that it fruited readily in artificial culture, but no other experiments have been recorded on the effects of environmental factors on fruiting of this fungus. In view of the small amount of data available, we feel justified in reporting these studies, although they are far from complete.

Materials and methods

The isolate of *P. arcularius* used was obtained from a dead Monterey Pine (*Pinus radiata* D. Don) at Marashioni, Kenya (9000 ft.). Before being used in the following experiments it had been cultured for about 9 months on 2 % malt agar, on which it fruited regularly.

Growth on malt agar at 15–20° C. appears first as a thin appressed mycelium which, after 2 or 3 days, assumes a thicker, white woolly texture. At this stage the mycelium may show considerable variation in growth rate and appearance, the latter varying from an even felted mat to an irregular tufted growth. At 14–18 days, a reddish brown stromatic mycelium begins to form over the oldest parts of the culture, while buff-coloured powdery streaks appear in the younger parts. It is from the latter that small, dark-brown, conical sporophore initials arise during the next 3–7 days; these elongate rapidly to a height of 5 to 20 mm. Towards the end of the period of elongation, the tip of the initial begins to flatten and pores appear on the under side; the colour of the pores eventually becomes

creamy white as the hymenium matures. The majority of spores germinate at once under moist conditions. Microscopic examination of the mycelium shows an increase of branching with age. Clamp connexions can be observed at all stages, but are rather more frequent in older mycelium. They become more difficult to make out with more profuse branching.

Cultures on test-tube slopes containing 10 or 15 ml. agar were used for all experiments. Inoculum for slopes was cut from plate cultures on 2 % malt agar with a 6 mm. diameter cork borer, care being taken to ensure uniformity of age and mycelial type within each experiment. Most experiments were carried out on the laboratory bench in normal daylight (essential to sporophore formation); when they were done elsewhere, this has been noted. Two per cent malt agar (made from 'Oxoid' malt extract) was used in all experiments except those designed to investigate effects of nutritional factors. Records of mycelial growth refer to the distance between the edge of the colony and the edge of the inoculum disk, along the length of the tube towards the base.

Experimental results

Genetical factors

Early observations showed that different isolates of *P. arcularius* varied considerably in their ability to produce sporophores in culture. In two experiments, therefore, single basidiospore isolations of the fungus were observed for sporophore production in culture. These isolates were obtained by streaking out a thick suspension of basidiospores in sterile water on plates of malt agar, following a method described by Dowson (1949). After incubation, well-isolated colonies were picked off the plates and tubed for observation. In both experiments nearly half the single spore isolates gave completely sterile cultures, while the rest formed either abortive or fully fertile sporophores (Exp. 1, 7 sterile cultures out of 13; Exp. 2, 15 sterile cultures out of 28).

It is possible that the capacity to form sporophores in culture depends on a single heritable factor. Alternatively, sterility might be due to heterothallism. To test the second alternative, eight sterile isolates were paired-off on 2 % malt agar. In none of these combinations was any tendency to form sporophores observed, and so it was concluded that sterility was connected with a heritable factor controlling the initiation of sporophores.

Effects of light

Intensity. In an early experiment the development of sporophores in six cultures exposed to normal daylight was compared with that in six others kept in the dark except for brief periods of daily observation. Over a period of 28 days those exposed to daylight gave rise to a total of fifty-one initials of which seven completed development, while corresponding cultures in the dark did not fruit but produced reddish brown stromatic mycelium in the older parts of the colony. Later, those cultures which had been kept in the dark were exposed to normal daylight, when they developed fertile sporophores. As for many other Basidiomycetes (Long & Harsch,

1918; Etter, 1928), light appears to be essential for fruit-body formation, although darkness results in total inhibition of development of the fructification, as opposed to inhibition only of pileus formation recorded by others (Galleymore, 1949; Buller, 1905, 1906).

An attempt was made to determine the effects of different periods of illumination on sporophore formation in culture. Three sets of tubes were set up in the constant temperature room (28° C.) and three in the laboratory; for each environment, one set was kept continuously in the dark except for brief periods of inspection, one was exposed to normal daylight for 8 hr. a day, and one was exposed to daylight by day and illuminated by a 60 W. pearl lamp at a distance of about 2 ft. by night. Recordings of sporophore formation made after 18 days (Table 1) showed an

Table 1. *Sporophore formation by* Polyporus arcularius *on 2 % malt agar under various conditions of temperature and illumination, after 18 days*

(Five replicates.)

	Mean fruiting per tube						
	No light		8 hr. day		24 hr. day		
Temperature conditions	Initials	Mature	Initials	Mature	Initials	Mature	Mean Initials + mature
Laboratory (17–20° C.)	0	0	1·5	0	4·0	1·5	1·2
Constant temperature room (28° C.)	0·7	0	1·6	0·7	4·0	1·0	1·3
Mean	0·3	0	1·6	0·4	4·0	1·3	—

increase approximately in proportion to the time the cultures were exposed to light. The two initials produced in the dark are thought to be in response to the brief daily exposure to light in the course of inspection. Plunkett (1953) has shown a very similar relationship for *Collybia velutipes*.

Wave-length. The effects of different coloured light on the formation of sporophores was then studied. Slope cultures grown at room temperature under normal daylight were screened as completely as possible by immersion in flat-sided glass jars containing solutions of copper sulphate, nickel chloride, or potassium dichromate, the strength of which was adjusted with the aid of a photographic exposure meter to give approximately the same intensity of transmitted light. A fourth jar containing water and indian ink, mixed to give the same intensity of transmitted (grey) light as the coloured solutions, was included in the series, and a fifth jar contained water alone. This experiment (Table 2) showed that blue light and day-

Table 2. *Effects of coloured light on production of sporophores by* Polyporus arcularius *in culture, after 20 days*

(Eight replicates.)

Screening solution	Approx. wave-length of transmitted light (A. units)	Mean fruiting per tube		
		Initials	Mature	Total
Potassium dichromate (orange)	5000–6800	0	0	0
Nickel chloride (green)	5500–6000	1·9	0·4	2·3
Copper sulphate (blue)	4300–5300	2·0	0·6	2·6
Dilute indian ink (grey)	—	1·3	0·4	1·7
Water (normal daylight)	—	2·0	0·5	2·5

light were equally effective in stimulating sporophore formation. Cultures grown in orange light showed the same characteristics as those grown in the dark. Effects of colour and intensity of illumination on mycelial growth were slight.

Nutrition

Acidity. An experiment was designed to determine the effects of a range of pH values on the formation of sporophores. A 2 % malt agar medium was made up with McIlvaine buffer solutions adjusted to six pH levels. Table 3 gives results obtained for mycelial growth and sporophore production, showing that both are markedly favoured by media made up with acid buffer mixtures. The limits for sporophore formation indicated by

Table 3. *Effect of* pH *range on growth and sporulation of* Polyporus arcularius

(Six replicates.)

Initial pH of medium	4·6	5·4	6·0	6·4	6·8	7·0
pH of medium after 19 days	4·8	4·7	4·9	5·2	6·6	6·8
Sporophores per tube (after 19 days)						
Initials	2·9	10·3	11·1	5·6	0	0
Mature	3·4	3·1	0	0	0	0
Linear mycelial growth (mm. after 13 days)	34	34	24	12	1	1

these results (pH 5·2–6·4) may not have a general application. Good sporophore formation has been observed on malt agar at pH 6·8 in other experiments, and it is possible that the high osmotic pressure and somewhat complex constitution of the buffered media has modified results in this experiment. Nevertheless, it may be concluded that alkaline media do not favour sporophore formation.

Nutrient concentration. This was investigated in three experiments; malt agar under similar conditions was used in all experiments, and all gave comparable results. The most comprehensive of these (Table 4) showed that increases in the malt concentration of the medium are accompanied

Table 4. *Effect of malt concentration on growth and sporophore formation by* Polyporus arcularius

(Ten replicates.)

Malt (%)	Linear mycelial growth (mm.) per tube after		Sporophores (mean no. per tube) after				Mean wt. sporophore tissue per tube after 24 days (g.)
			18 days		24 days		
	5 days	14 days	Initials	Mature	Initials	Mature	
0	0·5	1·5	0	0	0	0	0
0·1	4·3	7·5	0·2	0	0·4	0·1	Trace
0·5	7·0	12·5	0·7	0·2	1·0	0·2	0·001
2·0	10·8	19·1	2·9	0·2	2·5	0·9	0·063
5·0	12·4	22·6	3·9	0	3·5	0·5	0·243
10·0	10·1	18·1	3·4	0·6	2·2	1·1	0·375

by corresponding increases in linear mycelial growth and numbers of sporophores up to a value of 5 % malt. Between 5 and 10 % malt, linear growth of mycelium decreased but, as the thickness of the mycelial mat

was greatest at the 10 % level, it is possible that the greatest mass of mycelium was formed at the highest concentration of malt extract. Similarly, numbers of sporophores decreased between 5 and 10 % malt, but the fresh weight of sporophore tissue formed per tube was, nevertheless, greatest at 10 % malt.

At malt concentrations below 2 %, the sporophores were small and infertile. Above this concentration they were fully fertile, and at 10 % malt pilei had reflexed margins and abnormally profuse sporulation, the former being probably due to excessive formation of hymenium.

Sporophore initials showed a tendency to form round the edge of inoculum disks on media containing 0·5 and 1 % malt. It was thought that this might be due to the relatively high nutrient concentration in the inoculum, and a further experiment was made to determine the effect of

Table 5. *Relative effects of various malt concentrations in inoculum and substrate on mycelial growth and sporophore production of* Polyporus arcularius

(Five replicates.)

Inoculum concentration (%)	Substrate concentration (%)					
	0·5		2·0		5·0	
	Average linear mycelial growth after 3 days (mm.)					
0·5	6·5		9·0		9·5	
2·0	6·8		9·5		9·5	
5·0	7·4		9·5		10·0	
	Average numbers of sporophores formed per tube after 37 days					
	Initials	Mature	Initials	Mature	Initials	Mature
0·5	0·5	0	2·3	3·5	11·5	2·0
2·0	1·0	0	5·5	1·3	9·0	2·3
5·0	1·0	0·3	2·5	4·0	10·3	1·5

varying nutrient concentrations in inoculum and substrate on the mycelial growth and sporophore production. In this experiment, all combinations of three malt concentrations in substrate and inoculum, respectively, were compared (see Table 5). This shows that although the nutrient concentration of the inoculum can affect mycelial growth and fruiting, this effect is very small compared with that of the nutrient in the substrate. Where nutrient in the inoculum disk was relatively high, sporophores tended to form round it.

Nitrogen and growth factors. Early observations had shown that while *P. arcularius* readily formed sporophores on media containing malt, yeast extract, peptone or potato, none were formed on media based on carrot or combinations of carbohydrates and mineral salts. This suggested that growth factors and possibly a complex nitrogen source were necessary for fruiting in culture. In further experiments to investigate this a basal culture medium was used composed of 2 % agar, 0·1 % potassium dihydrogen phosphate, 0·05 % magnesium sulphate (hydrated) and a trace of ferrous sulphate. To this, nitrogen sources, carbohydrate, and growth factors were added as required.

The first experiment was designed to compare the relative importance of peptone ('Oxoid'), ammonium acetate, and sodium nitrate as nitrogen sources, with and without growth factors added in yeast extract. Dextrose was added to all media at 2 % and peptone at 0·5 % (0·065 % N), and other nitrogen sources were adjusted to give equivalent concentrations of nitrogen; yeast extract at 0·1 % was added to half the tubes. The results (Table 6) showed that in the presence of yeast extract sporophore initials

Table 6. *Effect of various nitrogen sources and yeast extract in a dextrose mineral agar on sporophore production by* Polyporus arcularius

(Eight replicates.)

Nitrogen source	0·1 % yeast extract	Linear mycelial growth after 4 days (mm.)	Sporophore production per tube after 28 days	
			Initials	Mature
Peptone	Present	9·3	2·5	1·0
	Absent	9·4	5·3	1·3
Ammonium acetate	Present	8·8	13·8	0·9
	Absent	8·4	0	0
Sodium nitrate	Present	11·1	5·3	0·5
	Absent	9·5	2·0*	0

* Very small initials.

became established with any nitrogen source. They matured on peptone medium, but formed only a few spores on nitrate and none at all on ammonium acetate medium. Without yeast extract only a very few tiny initials arose on the nitrate and none occurred on the ammonium media. These results show that complete sporulation of *P. arcularius* depends largely on one or more growth factors present in yeast extract and peptone. The various media were tested for pH before and at the end of the experiment, but no differences which might account for the results obtained were detected.

The effect of a range of concentrations of yeast extract in 2 % dextrose, 0·4 % sodium nitrate, plus basal salts was then tested in one experiment. The results (Table 7) showed a slight increase in mycelial growth rate with

Table 7. *Effect of yeast extract concentration in 2 % dextrose/sodium nitrate medium on mycelial growth and sporophore production of* Polyporus arcularius

Yeast extract (%)	Linear mycelial growth after 7 days (mm. per tube)	Sporophore formation after 30 days (no./tube)	
		Initials	Mature
0·000	18·9	2·0	0
0·001	19·3	2·1	0
0·020	20·7	1·4	2·0
0·050	21·3	1·8	0·8
0·100	26·4	1·7	0·8

increasing yeast extract concentration, and sporophore initials at all concentrations of yeast. However, at the lower yeast concentrations they remained very small and abortive. It is possible that they arose in response to traces of growth factors introduced with the inoculum disk. The opti-

mum percentage of yeast extract for fruiting appears to be about 0·02 %; Hawker (1942) has shown, for a range of fungi, that this value depends on the concentration of available carbohydrate. The effect on fruiting of various pure preparations of B group vitamins at a concentration of 5 μg./100 ml. in 2 % dextrose and basal medium was then tested. Distorted initials only were obtained in certain cultures, indicating that thiamin was possibly the only essential growth factor (Table 8).

Table 8. *Effect of certain B group vitamins on sporophore production by* Polyporus arcularius

Medium	Sporophore formation
Dextrose-nitrate basal medium alone	Nil
+0·1 % yeast extract	Distorted initials
+thiamin hydrochloride	Distorted initials
+pyridoxine	Nil
+riboflavine	Nil
+nicotinic acid	Nil
+all growth factors	Distorted initials
+0·05 % peptone	Distorted initials

C/N *ratio.* Three experiments were carried out to determine the effect of various proportions of dextrose and peptone on mycelial growth and fruiting. In each experiment, sporophore formation was inhibited at concentrations of nitrogen (as peptone) greater than 0·06 %, regardless of dextrose concentration up to 2 %, but the buff coloured powdery mycelium generally regarded as the precursor of sporophores appeared, nevertheless, in all the media.

The inhibition of sporophore formation at high peptone concentrations was thought possibly to be due to the accumulation of alkaline staling products and, to test this supposition, pH values of staled media from the third experiment of the series were determined by glass electrode. The results (Table 9) show that at the higher concentrations of peptone the

Table 9. pH *values of staled dextrose/peptone media*

	Nitrogen (%)			
Dextrose (%)	0·023	0·046	0·092	0·184
1·00	6·6	6·9	7·5	7·5
2·00	5·8	6·6	7·6	7·6

medium became sufficiently alkaline to inhibit sporophore formation completely. Plunkett (1953) obtained similar results with *Collybia volutipes*, but he pointed out that the action of staling products might not be entirely due to a change in pH. A final experiment has given results which may be interpreted on this basis. A range of nitrate nitrogen concentrations was set up in a medium comprising 2 % dextrose, 0·02 % yeast extract, and basal mineral agar (Table 10). A general decrease in mature sporophores formed by *P. arcularius* was found to follow increasing concentrations of sodium nitrate. No marked changes in pH occurred as these media staled and it seems possible that the reduction in numbers of sporophores may be brought about by effects unconnected with alkalinity.

Table 10. *Effect of varying sodium nitrate concentration on production of sporophores in culture by* Polyporus arcularius

Percentage nitrogen (as $NaNO_3$)	No. sporophores per tube after 48 days	
	Initials	Mature
0·03	3·4	2·4
0·07	2·8	0·6
0·13	4·8	0·6
0·26	1·6	0

We would like to thank Dr R. W. G. Dennis for his help in the identification of the fungus and for the information that he supplied relating to it, and Dr F. M. L. Sheffield for her helpful criticism on the manuscript.

REFERENCES

Badcock, E. C. (1943). Methods for obtaining fructifications of wood rotting fungi in culture. *Trans. Brit. mycol. Soc.* **26**, 127–32.

Buller, A. H. R. (1905). The reactions of the fruit bodies of *Lentinus lepideus* to external stimuli. *Ann. Bot., Lond.*, **19**, 127–138.

Buller, A. H. R. (1906). The biology of *Polyporus squamosus* Huds., a timber destroying fungus. *J. econ. Biol.* **1**, 101–133.

Dowson, W. J. (1949). *Manual of bacterial plant diseases.* London.

Etter, B. (1928). New media for developing sporophores of wood rot fungi. *Mycologia*, **21**, 197–203.

Galleymore, B. (1949). The development of fructifications of *Lentinus tuber-regium* Fries in culture. *Trans. Brit. mycol. Soc.* **32**, 315–317.

Hawker, L. E. (1942). The effect of vitamin B_1 on the concentration of glucose optimal for the fruiting of certain fungi. *Ann. Bot., Lond.*, **6**, 631–636.

Long, W. H. & Harsch, R. M. (1918). Pure cultures of wood rotting fungi on artificial media. *J. agric. Res.* **12**, 33–82.

Plunkett, B. E. (1953). Nutritional and other aspects of fruit body production in pure cultures of *Collybia volutipes* (Curt.) Fr. *Ann. Bot., Lond.*, **17**, 193–217.

(*Accepted for publication* 18 *February* 1956)

Trans. Brit. mycol. Soc. **40** (2), 221–231 (1957).

FACTORS AFFECTING THE GERMINATION OF SCLEROTIA AND MYCELIAL GROWTH OF *SCLEROTIUM ROLFSII* SACC.

By D. V. W. ABEYGUNAWARDENA and R. K. S. WOOD

Botany Department, Imperial College, London, S.W. 7

The effect of cultural conditions on mycelial growth and production of sclerotia by two isolates of *Sclerotium rolfsii* is described. The two isolates differed chiefly in numbers and weights of sclerotia produced, and in their ability to utilize lactose and nitrate nitrogen.

The isolates grew well on a variety of media and utilized a wide range of carbon and nitrogen compounds. On agar media, linear growth was most rapid at 30° C.; in liquid media best growth, as assessed by weight of mycelium, occurred between 20° and 30° C.; different optimal temperatures were obtained when cultures were harvested periodically over 24 days. For one isolate the optimum temperature for production of sclerotia was 10° C. The optimum pH for growth was near 3·0. Both isolates rapidly reduced the pH of media suitable for good growth to values between 2·5 and 4·0.

Sclerotia germinated well in thin films of distilled water. The effects of temperature, pH, humidity, soil extracts and nutrient solutions on germination are described. Germination was considerably reduced on or in soil, or when sclerotia were submerged in water, but when the soil or water was aerated, almost all sclerotia germinated.

Introduction

Sclerotium rolfsii Sacc. is a parasite of economic importance, principally in tropical and subtropical regions. It is soil-borne and has a wide host range; typically, it causes a damping-off of seedlings or a collar—or stem—rot of older plants. In spite of its economic importance, literature on the physiology of *S. rolfsii* in pure culture and on its pathogenicity is not extensive. Higgins (1927) studied the nutritional requirements of the fungus and the effect of temperature and pH on growth and production of sclerotia. Milthorpe (1941) worked along similar lines, and Johnson & Joham (1954) studied growth of the organism in synthetic nutrient solutions containing different sources of carbon.

Several workers have shown that the fungus exists in a number of distinct strains, which differ in their morphology and in pathogenicity towards different hosts. But few detailed comparisons have been made of the effect of nutritional and physical factors on the production of sclerotia and the growth of different isolates. There is also little information on the effect of environmental conditions on germination of sclerotia.

Past studies of the physiology of parasitism by *S. rolfsii* have been confined largely to establishing the nature of the substances, secreted by the fungus, which kill host tissue. Higgins (1927) failed to detect macerating enzymes in the culture filtrates he tested and concluded that the toxin produced was

oxalic acid; other workers, however, have provided good evidence that macerating enzymes are secreted.

In the light of the above, three lines of investigation have been followed in the present study. This paper presents the results of experiments on the mycelial growth and production of sclerotia by two isolates of the fungus under different conditions, and on the effects of different factors on the germination of sclerotia. A further paper will deal with the influence of nutrition on the production of extracellular pectic enzymes by the fungus.

Materials and methods

Two isolates of *Sclerotium rolfsii* were used: SR 4 isolated from tomato plants in Ceylon, and SR 7 from groundnut plants in the Gold Coast (supplied by the Commonwealth Mycological Institute). The isolates were morphologically distinguishable; SR 4 produced numerous spherical sclerotia whereas SR 7 produced a much smaller number of irregularly shaped sclerotia. After passage through their respective hosts, the two isolates were re-isolated and maintained on a glucose-peptone agar.

Standard 10 oz. medicine bottles containing 40 ml. media were used for liquid cultures, and 9 cm. Petri dishes containing 25 ml. media for agar cultures. Culture media were sterilized by autoclaving for 15 min. at 120° C., and were inoculated with disks (4 mm. diam.) from the advancing edge of a colony on 0·1 % peptone agar; five disks were added to liquid media and one placed mycelium downwards near the centre of each agar plate. Incubation was at 30° C. unless otherwise stated.

Daily increments of growth in agar cultures were measured along two diameters at right angles, and colony growth is given as the mean of not less than six such measurements. Growth in liquid culture was measured by harvesting the mycelium, generally before the formation of sclerotia, washing with cold water and drying at 70° C. to constant weight.

The production of sclerotia was assessed by harvesting after incubation for 3 weeks, washing with cold water, and counting before obtaining the dry weight. Large numbers of sclerotia were obtained by growing isolate SR 4 on a maize-meal/sand medium (2:98 by wt.); this was saturated but contained no free water. After inoculation and incubation for 3–4 weeks at 25° C., the surface of the medium became covered with masses of sclerotia, of uniform shape and size. Sclerotia selected for germination studies were surface-sterilized by soaking for 5 min. in a 1:400 dilution of saturated solution of bromine in water and then washed in several changes of sterile water and dried on sterile filter-paper at room temperature.

Germination of sclerotia was studied in the following ways:

(*a*) Ten sclerotia were placed on the surface of agar plates and incubated at 25° C.

(*b*) Five sclerotia were placed on or in soil or sand in glass phials (50 × 25 mm. diam.); after incubation for 1 week at 25° C. in a moist chamber, sclerotia were recovered and examined for growth.

(*c*) Areas 6 mm. square were marked on slides (3 × 1 in.) with a wax pencil; 0·05 ml. liquid was spread over each area as a thin film and a single sclerotium placed thereon. The slides were incubated in a moist chamber

at 25° C. Thin films of liquid were used because germination was erratic when sclerotia were completely submerged in liquid.

The results of experiments were analysed statistically; details of these analyses will not be given but differences of behaviour, between isolates or of the same isolate under different conditions, which are quoted in the text, are significant at $P < 0{\cdot}01$.

Mycelial growth and production of sclerotia

Different media

Vegetative growth and production of sclerotia by the two isolates were studied on a variety of media, the composition of which in g./l. is given below:

(*a*) Potato extract: peeled potatoes, 200.
(*b*) Carrot extract: peeled carrots, 300.
(*c*) Maize-meal: ground maize, 25.
(*d*) Malt extract: 15.
(*e*) Weindling's: glucose, 25; ammonium tartrate, 2·0; KH_2PO_4, 1·0; $MgSO_4.7H_2O$, 1·0.
(*f*) Czapek–Dox: sucrose, 30; $NaNO_3$, 2·0; KH_2PO_4, 1·0; $MgSO_4.7H_2O$, 0·5; KCl, 0·5; $FeSO_4.7H_2O$, 0·01.
(*g*) Glucose-peptone: glucose, 5·0; peptone, 5·0; KH_2PO_4, 1·75; $MgSO_4.7H_2O$, 0·75.
(*h*) Brown's: glucose, 2·0; asparagine, 2·0; K_3PO_4, 1·25; $MgSO_4.7H_2O$, 0·75.

To all synthetic media, 0·02 g. yeast extract and 1 ml. of the following minor element mixture were added per litre: $FeSO_4.7H_2O$, 0·1 g.; $CuSO_4.5H_2O$, 0·01 g.; $ZnSO_4.7H_2O$, 0·1 g.; $MnSO_4.4H_2O$, 0·1 g.; in 100 ml. water.

In liquid media both isolates grew luxuriantly in carrot extract and Weindling's medium, but very poorly or not at all in maize-meal extract and Brown's medium. Good growth was obtained in the other media. The mean values for the growth of the two isolates on the eight different media were 192 mg. (SR 4) and 179 mg. (SR 7). The initial pH values of the media were between 5·8 (glucose-peptone) and 7·7 (Brown's). The final pH values of media in which growth occurred were between 2·3 and 3·6. The poor growth of both isolates on maize-meal extract was surprising, in view of the richness of this medium.

On solid media the final pH values of the agar after growth were between 2·4 (Weindling's) and 4·7 (Brown's). Linear growth did not differ greatly on the different media; the rate of growth was highest on potato extract and lowest on Brown's medium. The production of sclerotia on the different media, and by the two isolates, was very different. No sclerotia were produced by either isolate on Czapek–Dox medium; both isolates produced the greatest weight of sclerotia on Weindling's medium. The mean weight of sclerotia produced by SR 4 on the eight media was approximately twice that produced by SR 7. There were, however, much greater differences in the numbers of sclerotia formed by the two isolates;

many more were produced by SR 4, e.g. on the glucose-peptone medium approximately 900 per plate were formed, compared with 130 by SR 7. The mean weight of sclerotia produced by SR 4 varied between 0·44 mg. (Weindling's) and 0·08 mg. (Glucose-peptone); corresponding figures for SR 7 were 2·3 mg. (Weindling's) and 0·12 mg. (potato-extract).

Carbon sources

The following basal medium was used: NH_4Cl, 1·16 g.; KH_2PO_4, 1·0 g.; $MgSO_4 . 7H_2O$, 1·0 g.; yeast extract, 0·02 g.; minor element mixture 1 ml. per litre. The carbon sources tested were: fructose, galactose, glucose, mannose, lactose, maltose, sucrose, starch, cellulose (filter-paper), sodium carboxymethylcellulose, pectin (citrus), mannitol, citric acid. Glucose was used at a final concentration of 2·5 % and the other materials in equivalent quantities.

With one exception, both isolates behaved similarly. The final pH values of the liquid media were generally below 3·0, and in no case below 2·0 or above 4·0. Growth was very good on fructose, glucose, mannose, maltose, sucrose, starch and pectin (300–500 mg.), relatively poor on the soluble cellulose (60 mg.) and on cellulose (assessed visually), and very poor on galactose, mannitol and citric acid (10–40 mg.). SR 7 produced three times as much mycelium (260 mg.) as SR 4 when lactose was used. Inability to use galactose has also been reported for a species of *Pilobolus* by Page (1952), and by Madelin (1953) for *Coprinus lagopus*. The poor vegetative growth on citric acid was probably related to the low initial pH (2·2) of the medium (see below).

Linear growth on agar media did not always correspond to dry weights of mycelia obtained in liquid culture, e.g. the growth rate in the presence of galactose was not much less than the highest obtained, which was on the agar containing sucrose. In these and other experiments, the rate of growth across agar was a poor measure of the amount of growth.

As in previous experiments, SR 4 produced a greater number and weight of sclerotia than did SR 7. In relating production of sclerotia on agar to production of mycelium in liquid media some striking differences were obtained. The weight of sclerotia produced by SR 4 on agar media was one-third to one-half the weight of mycelium produced in the corresponding liquid medium, when growth in this was very good. But when growth in liquid media was poor, SR 4 produced relatively a much greater weight of sclerotia, i.e. equal to or greater than the mycelium produced in liquid culture (Table 1).

TABLE 1. *Production of sclerotia by* Sclerotium rolfsii (*SR* 4)

Carbon source	Dry wt. (mg.) mycelium in liquid culture	Dry wt. (mg.) sclerotia on agar media
Glucose	510	210
Sucrose	450	240
Galactose	40	70
Sodium carboxymethyl-cellulose	70	60
Mannitol	20	60

In contrast, the weight of sclerotia produced by SR 7 was of the order of one-tenth to one-fiftieth of the weight of mycelium formed in liquid culture.

Neither isolate produced sclerotia when lactose was used.

Nitrogen sources

Peptone, asparagin, ammonium sulphate, ammonium nitrate, ammonium chloride, ammonium tartrate or sodium nitrate were used at a concentration of 0·03% nitrogen in a basal medium of glucose, 25·0 g.; KH_2PO_4, 1·0 g.; $MgSO_4.7H_2O$, 1·0 g.; yeast extract, 0·02 g., and 1 ml. minor element mixture per l.

In liquid media both isolates reduced the pH to final values between 2·0 and 2·6. Growth, which was good throughout (300–540 mg. per vessel), was in the order organic nitrogen, ammonium salts, sodium nitrate. SR 4 grew as well as, or slightly better than, SR 7, except in the media containing sodium nitrate, in which growth was some 30% less.

On agar, rate of linear growth was very similar on all media and the pH dropped to values between 2·0 and 2·2. SR 4 produced large numbers of sclerotia; the highest number per plate (*c.* 2500) was formed with peptone, and the lowest (*c.* 1300) with sodium nitrate. The dry weight of sclerotia produced was in the range 410 mg. (with ammonium tartrate) to 260 mg. (with ammonium chloride). Numbers and dry weights of sclerotia formed by SR 4 were between four and fifty times as great as those formed by SR 7, which produced a substantial weight (110 mg.) of sclerotia only on the medium containing peptone.

Temperature

Growth of both isolates was measured at 10°, 20°, 25°, 30°, 35° and 40° C., in the medium, described in the preceding section, with ammonium chloride. On solid media the growth of both fungi increased to a maximum at 30° C. and then declined; there was no growth at 40° C. No sclerotia were produced by SR 4 at 10° and 40° C.; the greatest weight was formed at 30° C.; the mean weight of single sclerotia decreased from 0·5 mg. at 20° C. to 0·08 mg. at 35° C. In contrast, SR 7 produced substantial quantities of sclerotia only at 10° and 30° C. (Table 2). At 10° C. the sclerotia remained white in colour for a considerable time but became brown when transferred to higher temperatures. These unusual results were confirmed by a second experiment.

TABLE 2. *Production of sclerotia by* Sclerotium rolfsii (*SR 7*) *at different temperatures*

Temperature (° C.)	Mean no. sclerotia/plate	Mean dry weight sclerotia (mg.)/plate
10	763	89
20	12	2
25	4	1
30	300	69
35	0	0
40	0	0

In liquid media, mycelia were harvested after 6, 12, 18 and 24 days at different temperatures. After 6 days' incubation, maximum growth of both isolates was at 30° C., but after 12 and 18 days the mycelium from cultures at 25° C. weighed 18–67 % more than did that produced at 30° C. After 24 days there was little difference in the weight of mycelium from SR 4 cultures at 10°, 20° or 25° C., but growth of SR 7 was greatest in the culture at 20° C. These differences in the optimum temperature for growth in cultures incubated for different periods may be explained in a number of ways, but these will not be discussed in the absence of further work.

Both isolates grew well at 35° C., but did not grow at 40° C.

Hydrogen-ion concentration

Earlier experiments showed that when the fungi grew well, pH values of media were rapidly reduced to values between 2·0 and 3·5. This production of acid made it difficult to study growth at different pH values without using relatively high concentrations of buffers. A citrate buffer was selected as one least likely to affect growth adversely, although citrate might have been used as a carbon source. It was used at a final concentration of 0·1 M in the sucrose/NH_4Cl medium given above; at low and high pH values, KH_2PO_4 and K_2HPO_4, respectively, were used; phosphate and buffer solutions were sterilized separately in each case. In spite of these precautions, the pH values drifted from the original values when conditions permitted good growth. This is shown in Table 3, which also gives the dry weights of mycelium from liquid media at different pH values.

TABLE 3. *Effect of* pH *on vegetative growth of* Sclerotium rolfsii

	SR 4		SR 7	
Initial pH	Final pH	Dry wt. (mg.)	Final pH	Dry wt. (mg.)
1·4	1·5	0	1·5	0
1·7	1·6	10	1·6	0
2·1	2·0	260	2·0	190
2·9	2·0	630	1·9	460
4·2	2·5	650	2·1	420
5·2	2·4	620	2·2	270
6·9	3·4	220	5·3	30
7·9	7·7	0	7·6	0
8·2	7·7	0	7·7	0
8·9	8·3	0	8·3	0

Nevertheless, a distinct effect of pH on growth was obtained. Both isolates behaved similarly, and grew best when the initial pH value was between 2·1 and 4·2. This fungus therefore has a remarkably low pH optimum for growth, probably near 3·0. Growth was greatly reduced when the initial value of the medium was below 2·1 or above 5·2.

On solid media, also, adequate control of pH was not obtained, except at the ends of the range, at which growth was poor. Linear growth and weight of sclerotia produced by both isolates was less affected by pH value of the medium than was weight of mycelium produced in liquid culture. This is illustrated by some of the data obtained with SR 4 (Table 4). Maximum growth and production of sclerotia by both isolates was in the range 2·8–4·2.

TABLE 4. *Linear growth and production of sclerotia by* Sclerotium rolfsii *on agar media at different* pH *values*

Initial pH	Final pH	Mean growth (mm.)/day	Mean no. sclerotia	Mean dry wt. (mg.) sclerotia
1·9	1·9	1	0	0
2·4	2·0	23	480	81
2·8	2·4	46	1810	247
4·2	3·6	46	1320	269
5·1	3·5	34	1230	233
7·8	3·6	12	1100	263
8·6	8·3	0	0	0

Light

Cultures were given one of the following treatments, in a constant temperature room at 25° C.

(*a*) Continuous light at 8 foot-candles (f.c.) (at the level of the surface of the medium, outside the culture vessel).

(*b*) Continuous darkness, except for an exposure of 10 min. to 8 f.c. after 4 days' growth.

(*c*) As (*b*) but exposed for *c.* 1 sec.

(*d*) Continuous darkness.

There were no significant differences in the weights of mycelia produced under treatments (*b*), (*c*) and (*d*), but some 15–20 % more mycelium was produced by both isolates in continuous light.

The dry weight of sclerotia formed by SR 4 was some 30 % higher in cultures kept in continuous light, and some four times as many were formed. Both weights and numbers of sclerotia produced by SR 7 under the different treatments were very low, and there were no significant differences between them.

GERMINATION OF SCLEROTIA

Sclerotia of SR 4 were collected from 3- to 4-week-old cultures and selected for uniformity of size and shape. The effect of various factors on germination of these sclerotia is summarized below.

Temperature

Sclerotia were placed on the surface of plates of Weindling's agar and incubated at 5°, 10°, 13°, 16°, 20°, 25°, 30°, 35° and 40° C. Each treatment consisted of five plates, each containing ten sclerotia (Table 5). The experiment was discontinued after 28 days; it seems likely, however, that at 10° C. sclerotia would have continued to germinate.

TABLE 5. *Germination of sclerotia of* Sclerotium rolfsii *at different temperatures*

Temperature (° C.)	
25, 30	All sclerotia germinated within 3 days
20, 35	All sclerotia germinated within 5 days
13, 16	All sclerotia germinated within 14 days
10	36 % sclerotia germinated within 28 days
5, 40	No sclerotia germinated within 28 days

Hydrogen-ion concentration

In these experiments there were a number of treatments, each consisting of three plates of Weindling's agar adjusted to particular pH values; ten sclerotia were placed on each plate (Table 6). Germination occurred most rapidly at pH 3·4, but was not greatly delayed at pH 2·6 and 4·4. At higher pH values germination was progressively delayed, but 46 % germinated after 96 hr. even at pH 7·7. The failure of sclerotia to germinate at pH 2·1 was surprising in view of the tolerance of the fungus to high acidity.

TABLE 6. *Effect of* pH *on germination of sclerotia of* Sclerotium rolfsii

	Percentage germination after			
pH	24 hr.	48 hr.	72 hr.	96 hr.
2·1	0	0	0	0
2·6	0	100	—	—
3·4	50	100	—	—
4·4	36	100	—	—
5·7	23	53	83	100
6·7	0	43	60	93
7·5	0	33	53	70
7·7	0	23	30	46

Nutrients

Higgins (1927) observed that sclerotia did not germinate well in distilled water. In the present work, however, sclerotia germinated well in distilled water if thin films were used. The effect of nutrients was studied, therefore, at pH 8·0, a value at which germination was delayed in the absence of nutrients. The following nutrients were used as 0·01 and 0·1 % solutions in thin films on glass slides; glucose, sucrose, sodium citrate, peptone and yeast extract. All nutrients at the lower concentration and sodium citrate at the higher concentration had little effect on germination, but the remaining nutrients at the higher concentration increased percentage germination of sclerotia after 40 hr. from 70 to 100 %.

Humidity

Sclerotia were attached to glass slides with a drop of gelatine which was allowed to dry, and were then placed in atmospheres maintained at different humidities by solutions containing suitable concentrations of sulphuric acid. Each treatment consisted of thirty sclerotia incubated at 30° C. At 100 % R.H., germination reached a maximum of 60 % in 4 days; at 99 % R.H., only 6 % of the sclerotia had germinated after 14 days, whereas at 97, 94 and 89 % R.H. there was no germination up to 14 days.

Soil moisture

In preliminary trials with unsterile soil, germination of sclerotia was observed only with difficulty, owing to the rapid and profuse growth of other soil fungi, particularly at higher moisture levels. Soil sterilized by

autoclaving was therefore used: a few days after sterilization, samples were brought to different water contents and placed in specimen tubes. Five sclerotia were placed on the surface or 20 mm. below, and ten tubes for each treatment were placed in a moist chamber at 25° C.; no correction for water loss was necessary (Table 7).

TABLE 7. *Effect of soil moisture on germination of sclerotia of* Sclerotium rolfsii

Water content of soil (% water holding capacity)	Percentage germination	
	Sclerotia buried	Sclerotia on surface
25	4	74
38	26	72
50	40	68
68	52	78
75	68	82
88	52	64
100	34	62

Up to 75% moisture holding capacity, germination of buried sclerotia increased with increasing moisture content; at higher moisture levels germination was reduced. Sclerotia on the surface germinated better than did buried sclerotia at all moisture levels; the differences were greatest at the two lowest moisture levels. Germination was again reduced at the two highest moisture levels.

Bedding material

The following materials were used:

(*a*) Medium loam, pH 6·5, water holding capacity 44 g. per 100 g. dry soil.

(*b*) John Innes potting compost, pH 5·8, water holding capacity 45 g.

(*c*) Coarse sand, pH 6·4, water holding capacity 19 g.

(*d*) Vermiculite (particle size 2–4 mm.), pH 7·5, water holding capacity 24 g.

These materials were passed through a 4 mm. sieve and the water contents adjusted to 75% moisture holding capacity; they were placed in specimen tubes with five sclerotia in each, on the surface of the soil or 20 mm. below; the depth of material below the sclerotia was 30 mm. in each case. Each treatment was replicated 10 times (Table 8). Except for Vermiculite, in which buried sclerotia germinated well, sclerotia on the surface germinated better than sclerotia which were buried. There was

TABLE 8. *Germination of sclerotia of* Sclerotium rolfsii *on and in different bedding materials*

Substrate	Percentage germination	
	Sclerotia buried	Sclerotia on surface
Soil	40	56
Potting compost	60	98
Sand	56	88
Vermiculite	86	88

considerably less germination with soil than with other bedding materials, particularly for sclerotia on the surface.

Soil-extracts

In view of the poorer germination of sclerotia in soil, further samples of the soil were extracted in various ways and the effects of these extracts on germination were investigated. Untreated soil, air-dried soil and soil dried at 70° C. for 24 hr. were extracted with sterile water; an amount equivalent to 50 g. on a dry weight basis was mixed with 100 ml. water and stirred for 4 hr. The mixture was centrifuged at 1000 r.p.m. for 10 min. and portions were tested with no further treatment, or after passage through a Seitz filter or a bacteriological sintered glass filter. Sclerotia were placed in films of these extracts and germination tested. None of the extracts had any effect on germination, which was uniformly good.

Aeration

In view of the results just described, the effect of aeration was investigated. Glass tubes (4 × 1 in. diam.) were filled with the soil used in the above experiments after it had been adjusted to a moisture content corresponding to 75 % moisture holding capacity. As before, five sclerotia were placed at or 20 mm. below the surface of the soil. The tubes were connected to a series of manifolds and moistened air was drawn through the soil at the rate of 0·3 l. per tube per min. A set of non-aerated tubes as kept in a moist chamber (Table 9).

TABLE 9. *Effect of aeration on germination of sclerotia of* Sclerotium rolfsii

	Percentage germination	
Treatment	Sclerotia buried	Sclerotia on surface
Aerated	93	97
Not aerated	52	72

Germination of sclerotia was considerably increased when the soil was aerated. As a sequel to these experiments, fifty sclerotia were placed in each of two muslin bags, which were suspended at a depth of 2·5 in. in bottles of water. Air was continuously bubbled through one bottle but not through the other; 92 % of the sclerotia suspended in aerated water germinated, but only 6 % germinated when the water was not aerated.

DISCUSSION

The two isolates used in this work differed primarily in their ability to produce sclerotia. The tomato isolate (SR 4) produced more sclerotia than did the groundnut isolate (SR 7); differences in numbers and weights of sclerotia were particularly pronounced when both isolates grew well. Only at 10° C. did SR 7 produce more sclerotia than SR 4; it was surprising that large numbers were produced by SR 7 at this temperature because none were formed at lower temperatures and relatively few appeared between 10° and 30° C.

With few exceptions, the amounts of vegetative mycelium produced by the two isolates under different cultural conditions were similar, but SR 7 produced three times as much mycelium as did SR 4 with lactose as the carbon source, and some 30 % more mycelium with sodium nitrate as the nitrogen source. The fact that the two isolates differed in their ability to use lactose may explain the conflicting reports by Higgins (1927), who stated that lactose was a poor source of carbon, and by Johnson & Joham (1954), who found it the best of twenty-three carbon sources tested. Here it is worth noting that the isolate used by Johnson & Joham did not grow on cellulose, whereas both SR 4 and SR 7 grew moderately well on cellulose and on sodium carboxymethylcellulose.

Growth of the two isolates was assessed either by weighing mycelium produced in liquid culture or by measuring rate of growth across an agar plate. The weight of mycelium produced gave the better measure of growth. This has been appreciated by many workers in the past, but linear growth across an agar surface is still widely used as a measure of growth. Assessing growth on weights of mycelium is also not without its dangers. This was illustrated by the results obtained when the fungus was grown in liquid media at different temperatures and the mycelia harvested at intervals over a 24-day period. After 6 days the greatest weight of mycelium was obtained at 30° C., but after 12 days the optimum temperature was 25° C., and for one isolate it was 20° C. after 24 days' growth. Time of sampling may obviously be important in this type of experiment.

The most important results emerging from the study of germination of sclerotia were that germination was reduced when sclerotia were placed on the surface of a fertile soil and reduced still further when they were buried at a depth of 20 mm. Water extracts of the soil did not affect germination on glass slides, but sclerotia on or below the surface germinated well when the soil was aerated. It seems possible that, in non-aerated soil, germination of sclerotia was reduced by an accumulation of carbon dioxide in the upper layers; the direct effect of carbon dioxide concentration on germination was not, however, studied.

A further point of interest was that even under unfavourable conditions such as low temperatures or high pH values, a substantial proportion of the sclerotia did germinate finally, although germination was greatly delayed.

REFERENCES

Higgins, B. B. (1927). Physiology and parasitism of *Sclerotium rolfsii* Sacc. *Phytopathology*, **17**, 417–448.

Johnson, S. P. & Joham, E. (1954). Some physiological notes on *Sclerotium rolfsii*. *Plant Dis. Rep.* **38**, 602–606.

Madelin, M. F. (1953). Factors influencing growth and fruiting of *Coprinus lagopus* Fr. Ph.D. Thesis, University of London.

Milthorpe, F. L. (1941). Studies on *Corticium Rolfsii* (Sacc.) Curzi (*Sclerotium rolfsii* Sacc.). I. Cultural characters and perfect stage. II. Mechanisms of parasitism. *Proc. Linn. Soc. N.S.W.* **66**, 65–75.

Page, R. M. (1952). The effect of nutrition on growth and sporulation of *Pilobolus*. *Amer. J. Bot.* **39**, 731–739.

(*Accepted for publication* 13 *April* 1956)

Trans. Brit. mycol. Soc. **40** (2) 232–236 ,(1957).

OCCURRENCE OF *RAMULASPERA HOLCI-LANATI* IN BRITAIN

By ELLIS GRIFFITHS

Department of Agricultural Botany, University College of Wales, Aberystwyth

(With Plate 9 and 3 Text-figures)

Ramulaspera holci-lanati (Cav.) Lind. is recorded for Britain. The morphology of the fungus on its host (*Holcus lanatus*) and in culture is described. The taxonomic position of the species is briefly discussed.

Introduction

Cavara (1893*a*) described, on *Holcus lanatus*, the fungus *Ovularia holci-lanati*, which Lindau (1907) later transferred to the spiny-spored genus *Ramulaspera* as *R. holci-lanati* (Cav.) Lind. Since then there have been no other records of this fungus, though several similar fungi (usually classified as *Ovularia*) have been reported on grasses both in Europe and in the United States (Sprague, 1948). None of these species has, however, been reported in this country, and all the collections at the Commonwealth Mycological Institute and the Royal Botanical Gardens, Kew, are of continental origin.

During the spring and autumn of 1954 and 1955 leaves of *Holcus lanatus* were found in the Aberystwyth area with eye-spot type lesions. Some of these bore a sparse crop of conidiophores and conidia, and the fungus was readily referable to *R. holci-lanati*. A short account of the fungus is presented here since it is a new record for the country and little is known of this and similar fungi on grasses.

Description

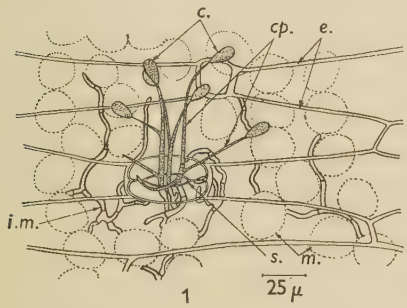
The fungus was found in two localities in the Aberystwyth district (Llanfarian and Tre'rddol). The distribution in both localities was limited, being restricted to very moist situations. It was never found on any grass other than *Holcus lanatus*, though it was carefully searched for on all the grasses (*Agrostis tenuis*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Holcus mollis*, *Molinia caerulea* and *Poa trivialis*) growing intermixed with infected plants of *H. lanatus*.

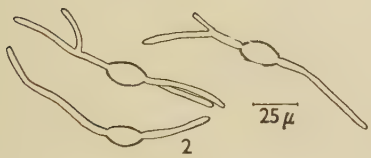
Only the leaves are infected, and on these, small dark brown spots with a yellow margin are produced (Pl. 9, figs. 1, 2). The spots are generally small, 1–2 mm. long by about 1 mm. wide, but occasionally up to 5–6 × 2 mm.

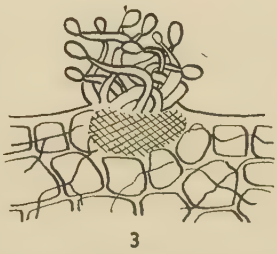
On freshly gathered material conidiophores and conidia are rare, but after incubation in a moist chamber overnight most spots produce a sparse crop of conidiophores. These arise from a branched intercellular mycelium and always emerge through the stomata. The conidiophores arise directly

from the mycelium; there is no development of a stroma in the substomatal cavity (Text-fig. 1).

The conidiophores, which are septate and sparingly branched are 80–100 × 2 μ. This is much shorter than the measurement given by Cavara (quoted by Sprague, 1948) which is 170 × 2 μ. It is, however, much longer than that given for any species of *Ovularia* on grasses, where measurements ranging from 20 to 40 μ are usually given.







Text-fig. 1. *Ramulaspera holci-lanati* (Cav.) Lind. Conidiophores and conidia on leaf of *Holcus lanatus*. (*c.*, conidia; *cp.*, conidiophores; *s.*, stomate; *i.m.*, intercellular mycelium; *m.*, mesophyll cells; *e.*, epidermal cells.)

Text-fig. 2. Germinating conidia (after 18 hr. at 20° C.).

Text-fig. 3. *Ophiocladium hordei* Cav. Redrawn from Cavara's original figure (Cavara, 1893*b*).

The conidia are produced at the tips of the branches of the conidiophores which taper to a very fine point. They are echinulate with a prominent hilum (Pl. 9, fig. 3) and measure 13–22 × 5–11 μ (mean 17·8 × 7·6 μ), being well within the limits given by Cavara (16–27 × 6–10 μ).

The conidia germinate readily, producing two or three germ tubes (Text-fig. 2). On leaves of *H. lanatus* very long germ tubes are produced which grow over the surface of the leaf for considerable distances before finally entering through a stoma. No evidence of entry other than through stomata was found.

On agar media, growth is of a staling type; the colony is white with abundant aerial mycelium and conidiophores, which arise from submerged hyphae, are formed over the whole surface of the colony. The conidia which are produced in culture are somewhat shorter and broader than those found on leaves (9–22 × 6–13 μ; mean 16·1 × 9·2 μ). They are generally produced terminally as on the leaves but occasionally laterally. In old cultures small black knots of hyphae were produced but these showed no signs of developing further.

The nutritional requirements of the fungus appear to be very simple.

Excellent growth occurred on a minimal medium (glucose and mineral salts) and addition of growth factors (yeast extract, nucleic acid and casein hydrolysate) produced no response.

Taxonomic position

R. holci-lanati belongs to a group of 'Ramularia-like' fungi on grasses whose relationships to one another are in doubt. The problem has been discussed by Sprague (1948), who has referred all the American collections to two species, viz. *Ovularia hordei* (Cav.) Sprague and *O. pusilla* (Ung.) Sacc. & D. Sacc. In doing this he widened the concept of *O. hordei* as originally defined and the present position may be briefly summarized as follows:

(1) *Ovularia pusilla* (Ung.) Sacc. & D. Sacc. (*Ramularia pusilla* Ung., *Caeoma pusillum* Bonord., *R. pulchella* Cesati, *Ovularia pulchella* (Ces.) Sacc.)

This was originally described on *Poa nemoralis* (Unger, 1833) and was later transferred to *Ovularia* by Saccardo (1906), who described it as

Hyphis erectis, subramosis, caespitulosis, exstomatibus exsilientibus; conidiis ovato-ellipsoideis, acrogenis, continuis, hyalinis, hyphae substriplo brevioribus.

No measurements are given, but Saccardo states that on checking Unger's figure the conidia are about 8–10 × 5–6 μ.

This fungus has subsequently been recorded on several other species of grasses (Sprague, 1946) and doubtful varieties have been described on *Lolium italicum* (Briosi & Cavara, 1889) and *Agropyron* (Davis, 1919). Taking these collections as a whole, however, all agree in having spores within the range 8–12 × 5–9 μ. No mention is made of the spore wall nor of the origin of the conidiophores—characters which are important in differentiating *O. hordei* and *R. holci-lanati*.

Five collections of *O. pulchella* are available at the Commonwealth Mycological Institute and the Royal Botanical Gardens, Kew. These were examined, and with one exception all showed spores within the range 8–12 × 5–7 μ, with smooth or faintly roughened walls (Pl. 9, fig. 7). The conidiophores were all within the range 25–65 μ long and in two cases where their origin could be observed they arose from internal hyphae and not from a substomatal stroma. The remaining collection of *O. puchella* was on *Festuca elatior* and showed spores 12·9 × 6·1 μ with an echinulate wall (Pl. 9, fig. 8). The conidiophores were 35 μ long but their origin could not be observed. Without this character a precise diagnosis cannot be made, but on the character of the spore this collection would seem to fit into Sprague's concept of *O. hordei*.

(2) *Ovularia hordei* (Cav.) Sprague (*Ophiocladium hordei* Cav., *Ovularia lolii* Volk., *O. baldingerae* Eliasson)

This was first described as *Ophiocladium hordei* on *Hordeum sativum* by Cavara (1893*b*) as follows:

'*Ophiocladium* nov.gen. Hyphae fertiles fasciculatae, anguineo-tortuosae; conidia acrogena, hyalina, continua.

'*O. Hordei* n.spec. Acervulus minutissimus, rotundis, albis in maculis linearibus arescentibus; hyphis fertilibus e stroma subepidermico albo orientibus, hyalinus, continuis, vel raro 1–2 septatis, simplicibus 20–30 × 3–4 μ; conidiis ovatis vel ellipticus, hyalinis 6–8 × 4·5 μ.'

The principal diagnostic features—the tortuous conidiophores arising from a subepidermal stroma are shown in Text-fig. 3. Sprague and others have reported similar material though with somewhat different spore measurements on grasses in the United States (Sprague, 1946; Davis, 1937; Greene, 1942). Some collections, however, do not fit at all well into *O. hordei* as defined above. These have temporarily been included in *O. hordei* on the basis of the tortuousness of the conidiophores and an evident spore wall. Two collections have been singled out for special comment by Sprague; one on *Phalaris arundinacea* with spores 11–15·5 × 6–7 μ, the other on *Festuca kingii* with spores 16–22 × 9·5–10·5 μ. In the *Phalaris* collection faint muriculations were seen on the spore wall and these were more pronounced in the collection from *F. kingii*. This latter collection would seem to agree well with *R. holci-lanati* as far as spore characters are concerned; it differs, however, in the length of the conidiophores and unlike the Aberystwyth collection the conidiophores arise from a group of cells 14 × 14 μ.

It is evident that Sprague's collection on *F. kingii* comes very close to the collection on *Holcus lanatus* referred here to *Ramulaspera*. The fact that in the latter there is no tendency to tortuousness in the conidiophores and no subepidermal stroma precludes its inclusion in *O. hordei*.

That these fungi may all belong to one variable species appears very likely, but the evidence at present available does not appear to warrant their unification and Sprague's decision to maintain *O. pusilla*, *O. hordei* and *R. holci-lanati* as separate species seems justified.

I am indebted to the Directors of the Commonwealth Mycological Institute and the Royal Botanic Gardens, Kew, for permission to examine collections of *O. pulchella*. Herbarium specimens of *Ramulaspera holci-lanati* have been sent to both these institutions and a culture to the C.M.I.

REFERENCES

Briosi, G. & Cavara, F. (1889). *I funghi parassiti delle piante coltivate od utili*. Pavia.

Cavara, F. (1893*a*). *Fungi Longobardiae exsiccati sive mycetum specimina in Longobardia collecta, exsiccati et speciebus novis vel criticis, iconibus illustrata fasc.* III, no. 144. Ticini.

Cavara, F. (1893*b*). Ueber einige parasitische Pilze auf dem Getreide. *Z. PflKrankh.* **3**, 16–26.

Davis, J. J. (1919). Notes on parasitic fungi in Wisconsin, VI. *Trans. Wis. Acad. Sci. Arts Lett.* **19**, 705–715.

Davis, J. J. (1937). Notes on parasitic fungi in Wisconsin, XX. *Trans. Wis. Acad. Sci. Arts Lett.* **30**, 1–15.

Greene, H. G. (1942). Notes on Wisconsin parasitic fungi. *Trans. Wis. Acad. Sci. Arts Lett.* **34**, 83–98.

Lindau, G. (1907). In Rabenhorst, *Kryptogamen Flora von Deut., Oesterr., und Schweiz*, 2 Aufl., **1**: Pilze (Abt. 8). Leipzig.

Saccardo, P. A. (1906). *Sylloge Fungorum*, **18**, 531.

Sprague, R. (1946). Additions to the Fungi Imperfecti on grasses in the United States. *Mycologia*, **38**, 52–64.
Sprague, R. (1948). Some leafspot fungi on Western Gramineae. III. *Mycologia*, **40**, 294–313.
Unger, F. (1833). *Die Exanthema der Pflanzen*. Vienna.

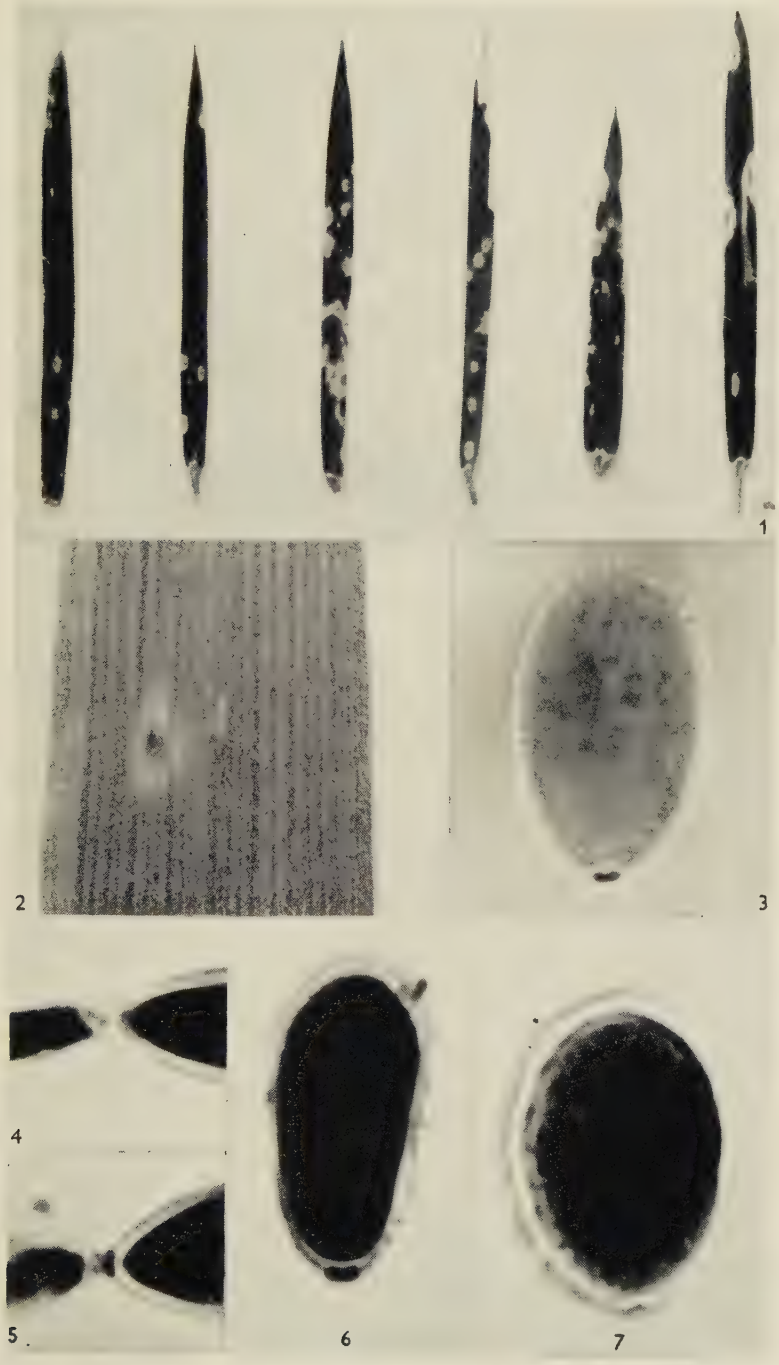
EXPLANATION OF PLATE 9

Figs. 1–7. *Ramulaspera holci-lanati* (Cav.) Lind.

Fig. 1. Leaves of *Holcus lanatus* showing spots (× 0·8).
Fig. 2. Leaf spot (× 12).
Fig. 3. Conidium (× 3000).
Figs. 4, 5. Stages in abscission of conidia (× 4000).
Fig. 6. Conidium of *Ovularia pulchella* (Ces.) Ung. from *Festuca elatior* (× 4000).
Fig. 7. Conidium of *Ovularia pulchella* (Ces.) Ung. from *Lolium italicum* (I.M.I. 7256) (× 4000).

(Figs. 4–7 are from preparations stained with cotton blue.)

(*Accepted for publication* 30 *March* 1956)



(*Facing p.* 236)

Trans. Brit. mycol. Soc. **40** (2), 237–262 (1957).

STUDIES ON THE OCCURRENCE AND ACTIVITY OF FUNGI IN A WHEAT-FIELD SOIL

By J. H. WARCUP

Waite Institute, Adelaide, South Australia

(With Plate 10 and 3 Text-figures)

The occurrence and activity of fungi in a wheat-field soil has been investigated by plating techniques and by direct isolation. Over 210 fungi have been isolated. A high proportion of the fungi obtained by hyphal isolation are species which were rare or absent from dilution plates. In contrast to dilution methods, where the most abundant fungi isolated were species of *Penicillium*, *Rhizopus*, *Mucor*, *Cladosporium* and *Fusarium*, many fungi obtained from hyphae remain sterile in culture. *Rhizoctonia solani*, *Ophiobolus graminis*, *Rhizoctonia* spp. and several Basidiomycetes were obtained by hyphal isolation.

Little difference in fungal count has been found between samples collected at 1 and 3 in., but significantly fewer fungal units were obtained from a subsurface horizon at 6 in. deep. Throughout the year fungi showed a well-marked periodicity, with highest numbers of viable units in the soil during the wet winter period. Both viable spores and viable hyphae were present in low numbers during the dry summer period; some fungi, however, survived the summer as hyphae. Bacterial and Actinomycete counts also showed a similar seasonal periodicity. Cultivation and incorporation of wheat residues with the soil greatly increased fungal numbers and activity, particularly in the neighbourhood of the residues. Bacterial numbers likewise increased greatly after cultivation, but Actinomycetes showed comparatively little change in number. The occurrence and activity of specific fungi are discussed. The data indicate that many species have very short periods of activity in the soil, but are always present.

Introduction

Since Adametz in 1886 first isolated fungi from soil, numerous studies have been carried out on the occurrence of various genera and species of fungi in soil. Extensive floristic lists have been prepared for many soils and such data used to compare fungal occurrence in different soil types, in different soil horizons and under different crops or vegetation. While much information has been accumulated, there are, however, few data on growth and activity, as distinct from mere occurrence of fungi in soil. Chesters (1949) has aptly remarked that so far only a very indistinct picture of the fungus at work has been obtained.

Much of the difficulty has centred around the fact that while fungi may exist in soil either as mycelium or spores, direct observation methods give little information as to the identity of the fungi observed, and cultural methods fail to discriminate between currently active mycelium and resting bodies (Garrett, 1955). Harley & Waid (1955) have clearly re-stated this problem by saying that: 'One of the first requirements for an ecological study of soil organisms is that the methods must distinguish between organisms which are vegetatively active and playing a part in the soil

processes and those which exist in a dormant or inactive form as spores and other propagules.'

This paper is part of a programme of work to investigate the activity of fungi in soil. The aim of this investigation has been twofold: to compare three different methods, the dilution plate method, the soil plate method, and the hyphal isolation method (Warcup, 1955*a*) for the isolation of fungi from soil; and to use the information obtained from these and supplementary methods to study the occurrence and activity of fungi in a wheat-field soil throughout the year.

The soil studied

The investigation was carried out in a wheat-field at the Waite Institute, Adelaide, South Australia. The Institute lies in a region with a Mediterranean type of climate, the features of which are a hot dry summer and a cool wet winter. Seasonal rains usually commence in March–June and conclude in September–December. the average annual rainfall being 24·6 in.

The soil investigated is a red-brown earth of the Urrbrae series (Piper, 1938). It has a silty loam texture with a pronounced fine sand fraction; mechanical analysis of the surface soil giving 5 % coarse sand, 44% fine sand, 24 % silt and 20 % clay. The surface soil, 0–6 in., has a characteristic brown colour due to the presence of organic matter, but the colour changes to reddish brown in the subsurface soil, 6–12 in., and the deep clay is red. The soil has a slightly acid reaction, pH varying from 6·1–6·5 in the surface horizon to 6·6–7·0 in the subsurface soil.

The marked seasonal incidence of rainfall allows great variation in soil moisture throughout the year, varying from below wilting point for most of the summer dry period to field capacity or above for some weeks during a wet winter. Indirect determination of wilting point at 15 atm. with a pressure membrane apparatus gave a water content of 6·7 % and field capacity (100 cm. water pressure) of 18·1 %. When dry the soil becomes very hard and compacted.

The field studied was in pasture in 1951, fallowed in 1952 and then had three successive wheat crops (1953–1955). Yield was high for the first two years but was less in the third year when take-all was present in the crop. Soil fungal analyses were made at fortnightly to monthly intervals from March 1954 to September 1955. Many supplementary samples were also collected.

Collection of soil samples and isolation of fungi

For the collection of soil samples a small profile pit was dug and sterilized specimen tubes (3 × 1 in.) pushed horizontally into a prepared face. Samples were collected at depths of 1, 3 and 6 in., the first two samples being above and the third below the maximum depth of tillage. Duplicate samples were also collected for determination of water content. During the summer when the soil was hard and compacted, soil blocks were obtained at the appropriate depths and later subsampled in the laboratory. Fungal analyses by dilution plate, soil plate and hyphal isolation methods were usually made on the day of collection of soil samples.

Plates were prepared at a dilution of 1/5000, 5 g. of soil being added to sterile water to give an initial dilution of 1/50, which was then raised to 1/5000 in one step. Three replicate flasks of 1/5000 were prepared and three plates poured from each flask, a total of nine plates per sample. For counting bacteria and Actinomycetes three further plates were prepared at a dilution of 1/250,000 and poured with soil agar (Timonin, 1940). Soil plates were prepared as described by Warcup (1950), six replicate plates being prepared from each sample. Both soil plates and dilution plates were poured with Dox + yeast agar (pH 4·2–4·4), incubated at 25° C., and examined at intervals over a period of 2–4 weeks for the recording or isolation of fungi. Bacteria and Actinomycetes were counted after 10 days at 25° C. Hyphal isolation plates (Pl. 10, fig. 1) were prepared as described by Warcup (1955*a*), two subsamples, each of approximately 1·5 g. being examined from each depth. About 150–200 hyphae per sample were isolated, except for the 6 in. samples where hyphae were less abundant. During the summer dry period hyphae were also less abundant in samples from all depths. Hyphal isolation plates were incubated at room temperature and examined daily under a microscope for fungal growth. Plates were kept 8–10 days, since some hyphae were found to be very slow in commencing growth.

Slow-growing colonies on hyphal isolation plates were prone to be overgrown by contaminating bacteria or by Actinomycetes growing from the surface of the original hypha or from attached humus particles. Sometimes removal of the contaminated colony in a small block of agar to a sterilized wheat straw facilitated isolation of the fungus, since many of the fungi grew better on straw than did the contaminants. All cultures were kept on Dox + yeast agar unless growth on that medium was unsatisfactory, in which event other media such as malt agar (Raper & Thom, 1949), carrot-potato agar (Langeron, 1945), starch-peptone agar (Raper, 1940), or natural substrates such as sterilized wheat straws, leaves or soil were used. A few fungi did not grow satisfactorily in culture and were soon lost.

Direct observation of soil, either in the field or in the laboratory, proved a useful supplementary method for the examination of fungi in soil. Kubiena (1938) has developed a special microscope for direct observation of undisturbed soil in the field, but in this study soil blocks were examined for the presence of fungi under a binocular dissecting microscope in the laboratory. Freshly collected blocks of undisturbed soil were repeatedly broken apart and the exposed surfaces examined; cut surfaces are unsatisfactory for observation (Kubiena & Renn, 1935). Direct observation showed hyphae, rhizomorphs, and sclerotia of fungi; fructifications were rarely found.

Comparison between methods of fungal isolation

Occurrence of fungi in soil

It soon became apparent that whereas the dilution and soil plate methods give essentially the same picture of the fungal flora, hyphal isolation gives a very different one. This is shown in Table 1, recording the most abundant fungi isolated by each method. Data for the dilution and hyphal isolation

methods record total number of colonies; for the soil plate method, where individual colonies are not counted, the total number of soil plates on which a fungus occurred is given. Table 1 shows that the most abundant fungi isolated by the dilution plate method are similar to those isolated by soil plates, although there may be differences in relative abundance. Most

Table 1. *The most abundant fungi in wheat-field soil, as recorded by dilution plate, soil plate and hyphal isolation methods*

Method of isolation ...	Dilution plate			Soil plate			Hyphal isolation		
Occurrence as ...	Total no. colonies			Total no. plates			Total no. colonies		
Depth of sample (in.)...	1	3	6	1	3	6	1	3	6
Penicillium W2	2405	2161	498	184	225	195	0	0	0
P. urticae	2247	103	1	87	32	12	0	0	0
?*Scopulariopsis* W21	476	683	233	118	159	149	0	0	0
Cladosporium herbarum	476	196	2	70	28	6	0	11	0
P. purpurogenum	394	421	228	122	73	64	0	0	0
Rhizopus arrhizus	367	261	3	158	83	20	1	5	0
Penicillium W3	245	314	141	98	125	116	0	0	0
P. lilacinum	164	203	116	78	106	110	0	0	0
Mucor W13	102	47	0	110	52	17	0	0	0
Penicillium W25	44	104	178	13	36	94	0	0	0
Fusarium oxysporum	223	164	31	182	161	58	16	5	0
Thielavia W16	81	96	30	107	127	84	1	0	0
Mortierella spp.	119	46	38	100	114	131	27	4	0
T7 (sterile culture)	13	5	2	0	0	1	167	49	10
T18 (sterile culture)	0	0	0	0	0	0	155	73	0
T49 (sterile culture)	0	0	0	1	0	0	139	30	3
T47 (sterile culture)	0	0	0	0	0	0	72	67	4
T69 (sterile culture)	0	0	0	0	0	0	70	48	9
T58 (sterile culture)	2	0	0	3	0	3	64	44	134
T6 (sterile culture)	8	7	5	4	0	20	41	88	31
Rhizoctonia T115	0	0	0	0	0	0	19	93	0
T62 (sterile culture)	0	0	0	0	0	0	50	83	0
T13 (sterile culture)	0	0	0	0	0	0	4	12	56
T9 (sterile culture)	0	0	0	0	0	0	12	34	36
Total no. colonies	7965	5638	1899	—	—	—	1585	1225	331
Total no. species	87	91	75	70	76	86	68	55	18

of these fungi, however, were rarely isolated by hyphal isolation; moreover, the most abundant fungi isolated from hyphae were rare or absent from the lists obtained from dilution or soil plates. The complete list (Appendix) of which Table 1 is a part, shows that sixty of the ninety-four species obtained by hyphal isolation were not recorded on dilution or soil plates. While some fungi isolated from hyphae were rare and may have been missed by chance by the other two methods, other species, as shown in Table 1, were abundant. Thus besides giving data on which fungi in a soil are present as mycelium, the hyphal isolation method records a large group of fungi that are neglected by the dilution- or soil-plate methods. This is contrary to opinions that the plate method enables a more or less accurate determination of the fungus population of the soil (Waksman, 1944; McLennan & Ducker, 1954). Thornton (1956) has recently commented that the soil-plate technique is unable to give a true indication of the fungal flora of natural grassland soils in New Zealand, since it gave no evidence of *Rhizoctonia*, which he found commonly by his screened immersion-plate technique.

While Table 1 shows that a higher total number of species was recorded from dilution than from soil plates, in any particular sample soil plates usually gave a higher number than did dilution plates. This difference in the number of species obtained by the two methods was most marked with subsurface samples, where soil plates often recorded twice the number on dilution plates, was less marked with surface samples, and if *Pythium* or *Trichoderma* was present was likely to be reversed, since both genera contain fast-growing species that may swamp other fungi; both were isolated more frequently from soil than from dilution plates. Besides these differences in the number of species obtained, several fungi, including *Absidia butleri*, *Cunninghamella*, *Penicillium* W4, *Trichoderma viride* and species of *Aspergillus*, *Pythium*, *Mucor*, and *Mortierella*, were usually more abundant on soil than on dilution plates. A species of *Melanospora* that showed synergistic growth, and was not obtained in pure culture, was of frequent occurrence on soil plates but was absent from dilution plates. Whether the difference in results obtained by the two methods can be attributed mainly to the difference in the dilutions used (dilution plates 1/5000, soil plates approximately 1/250) or whether other factors, such as a possible failure of some fungi to enter into soil suspensions, are also important is not known. Angell (1954) has shown that species of *Pythium* are usually more abundant in soil residues than in soil suspensions and this is also my experience; however, since published data (Angell, 1954; Warcup, 1952) suggest that *Pythium* spp. are usually present in soil as less than 1000 colonies per g., dilution plates prepared at high dilution again tend to miss them. In general, the data suggest that soil plates tend to favour medium to fast-growing fungi present in soil in relatively low numbers.

Table 1 also shows that while the abundant fungi isolated from dilution and soil plates are species of *Penicillium*, *Cladosporium*, *Rhizopus*, *Mucor* and *Fusarium* (genera frequently recorded from soil), many of the abundant fungi obtained by hyphal isolation remain sterile in culture. This is further emphasized in the Appendix. Examination of this list shows that about 70 % of the fungi isolated from hyphae have remained sterile in culture. Eight of these have clamp connexions and can be classed as Basidiomycetes, four are species of *Rhizoctonia*, several produce either sclerotia or chlamydospores but no other spore-form, and many are slow-growing with dark brown or black hyphae. Chesters (1949) considered that species of *Pythium* and *Mortierella*, the higher Basidiomycetes and a galaxy of darker Hyphomycetes are the striking omissions from the fungal flora usually reported from soil. Harley & Waid (1955) have recently shown that on living root systems cleansed of fungal spores by prolonged washing, many sterile mycelia and mycelia slow to produce spores, which are not obtained when unwashed root surfaces or dilutions of washings from root surfaces are cultured, occur. It is also interesting to recall that the fungi seen on Rossi-Cholodny slides are frequently sterile.

While these data show that hyphal isolation records many fungi not usually isolated by soil or dilution plates, it is also true that some fungi, notably species of *Penicillium*, were not isolated by this method. It is relevant here to consider how selective the hyphal isolation method may be. It is considered that the method, which consists in visual examination under

low magnification of soil residues for the detection and isolation of hyphae, may be selective for (*a*) hyphae of large diameter, (*b*) mycelia that do not fragment readily, and (*c*) fungi with dark-coloured hyphae. While large hyphae are easy to see and to isolate, in this soil their number was usually small and it was possible to guard against isolating more than a representative sample of them. Hyphal masses, either large or small, are usually easily found and provide the majority of the fungi isolated. Hyphae in such masses were very variable in size, ranging from 1–2 to 5–6 μ or more in diameter. Such hyphal masses often contained the hyphae of more than one species of fungus. Dark-coloured hyphae are more easily seen than hyaline ones; however, search for the latter is often made easier because of the fine mineral and humus particles attached to them (Thom, 1927).

Hyphal isolation may tend to neglect (*a*) hyphae that fragment readily, (*b*) fine single hyphae, (*c*) hyphae in large humus particles or grosser organic fragments in the soil, (*d*) hyphae closely adherent to or occurring in plant roots. Whether any fungi occurring in soil have mycelium that fragments easily is not known; there is evidence that fine unbranched hyphae occur in soil. There is considerable evidence that grosser organic fragments and plant roots are both important 'ecological niches' in soil which may be missed by the hyphal isolation method. It is also possible that hyphae of some fungi may be mainly removed in the suspension and thus not remain with the heavier soil residues during the preparation of hyphal isolation plates. To investigate this possibility, the suspension was passed through a 50 μ pore diameter bronze gauze sieve, and a representative sample of the hyphae retained plated-out in the usual manner but, in limited studies, no consistent differences have been noted between species isolated from suspension or residue. Further evidence against the view that fungi in the suspension may be different from those in the residue is that fungi isolated from hyphae occurring on dilution plates (Warcup, 1955*b*) are also similar to those isolated by hyphal isolation. Another selective element of the method that is not specifically considered here is that not all fungi present as hyphae in soil may be able to grow on the medium used for the isolation plates (Warcup, 1955*a*).

Information on the ability of the hyphal isolation method to isolate *Penicillium* from soil was sought by inoculating sterilized soil with spores of *Penicillium* W2 or *P. urticae*, incubating for 2–3 days and attempting hyphal isolation in the usual manner. Mycelia were abundant in the sterilized soil, and although hyaline, were readily isolated and grown on the isolation plates. Whether growth of *Penicillium* in sterilized soil is comparable with growth of the fungus in untreated soil in the field is doubtful, but the result does indicate that if abundant young active mycelium is present in soil it could be isolated by the hyphal isolation method. Further data on the occurrence and activity of *Penicillium* in the wheat-field soil are considered in the second part of this paper.

Activity of fungi in soil

So far the comparison between methods of isolation has been concerned with the occurrence of fungi; there is also the important question of the activity of fungi in this soil. The main basis for studying activity has been

the data obtained by the hyphal isolation method. If carried out with strict precautions against possible contamination from other fungal units that may be present, this method does indicate species that are present as hyphae in soil, though the presence of viable hyphae need not be synonymous with active growth in soil. Some fungi have been found present as hyphae in soil well below wilting point during the summer dry period when it seems unlikely that active growth could be proceeding. Data on activity have also been obtained by direct observation of soil.

Table 2. *Origins of fungal colonies developing on dilution plates prepared from ten samples of wheat-field soil*

Sample no.	Moisture content, % dry wt.	Percentage colonies developing from: Spores	Hyphae	Humus particles	Unclassified	No. colonies per g. air-dry soil (× 1000)
33	22·1	78	1	15	6	145
34	24·5	77	2	16	5	190
35	21·4	80	3	13	4	150
36	20·1	70	5	21	4	155
37	18·0	82	2	15	1	180
38	2·5	70	0	30	0	45
38a	2·1	54	0	42	4	75
39	2·5	62	0	38	0	40
39a	2·7	56	0	44	0	45
40	2·6	70	0	30	0	35

While hyphal isolation and direct observation give data on activity of fungi in soil, there still remains the important question of exactly what, spores or hyphae or what proportion of each, the soil and dilution plate methods measure. With data from this same wheat-field Warcup (1955*b*) has shown that the majority of the colonies isolated on dilution plates originated from spores, even when hyphal isolation showed abundant fungal growth in the soil. Further data on the origin of colonies developing on dilution plates prepared from the wheat-field soil are given in Table 2. The results substantiate the earlier data and also show that colonies from hyphae were not recorded from soil samples collected during the summer dry period. While little information was obtained for soil plates, the inference is that they, like dilution plates, predominantly record fungi present in the soil as spores; it is not certain that fungi isolated by this method originate from hyphae. These data show that unless an analysis of the unit of origin of individual colonies is carried out, no information on the current activity of fungi in soil is obtained by either the dilution or soil plate methods.

Further illustration of the different information obtained by soil and dilution plate methods compared with that from hyphal isolation is shown in Text-figs. 2 and 3, where all data obtained for twelve different fungi in the wheat-field soil are given. If we consider the data for *Rhizopus arrhizus* (Text-fig. 2C), we see that both soil and dilution plates record this fungus from most samples of surface soil throughout the year; hyphal isolation, however, recorded the fungus only twice, each time during the early stages of decomposition of residues after cultivation of the field. Direct observa-

tion showed that comparatively widespread growth of *Rhizopus* occurred in the soil on these occasions, and also that localized activity occurred at other periods of the year. The first subsidiary period of activity occurred in October 1954, when occasional hyphae and sporangia of *Rhizopus* were found in the soil around the bases of ripening wheat plants; further growth occurred after the heavy summer rain in February 1955 when sporadic growth occurred from partly buried stubble at the soil surface. It is interesting to note that on both occasions hyphae were not seen below half an inch in the soil, which means that even if the growth had been widespread, it would have been missed by normal sampling at 1, 3 and 6 in. deep. After the major period of activity of *Rhizopus* following cultivation in 1955, further growth of the fungus was noted in June when hyphae were found in the soil, in all cases growing from germinated wheat grains. The vegetative phase of *R. arrhizus* in soil appears to be of short duration, since young and mature sporangia were found in the soil 3 days after the February rain, and also all signs of hyphae had disappeared from the soil 4–6 weeks after the major period of growth on buried stubble. During periods of activity sporangia were formed profusely in soil, particularly in larger soil cavities such as worm tunnels (Pl. 10, fig. 4) and insect galleries; if formed in restricted space the sporangiophores were usually shorter and often coiled. Sporangia also appeared to be rapidly decomposed in the soil.

These results show that whereas *Rhizopus* had limited periods of activity in this soil, dilution and soil plate methods recorded its presence continuously throughout the year. The data also indicate that high counts obtained by dilution plates cannot be correlated with current activity of the fungus in the soil, though activity and high count may coincide. All examinations of origins of colonies so far made show that even where activity and high count coincide, it is the spore component and not the mycelial one that is recorded on the dilution plates. It is possible, however, that this may not be true for other species in the soil.

Though this discussion on fungal activity in soil has been largely restricted to *Rhizopus*, the data suggest that many fungi recorded frequently on soil and dilution plates throughout the year may have limited active growth in the soil. Text-figs. 2 and 3 also show other fungi of which the presence and activity in soil is scarcely recorded by soil or dilution plates.

The results obtained for *Rhizopus* by direct observation, showing that a fungus may be active in soil in very restricted areas or for short periods, suggest that it may be difficult to investigate fully the activity of a fungus in soil.

Ecology of the fungal population in the wheat-field soil

In this section data obtained by all methods of investigation are used to discuss the occurrence and activity of fungi in the wheat-field soil. Fungal counts were obtained by the dilution-plate method and are considered for the reasons previously discussed to record predominantly the number and kind of non-active fungal units in the soil. Since the data obtained from soil plates are similar to those obtained from dilution plates they are not given in detail, but are used to supplement other data where necessary. Data on fungal activity, unless otherwise stated, have been gained by

hyphal isolation. Counts of bacteria and Actinomycetes obtained from dilution plates of soil agar are also discussed. Text-fig. 1 shows the counts of bacteria, Actinomycetes and fungi; counts were obtained at depths of 1, 3 and 6 in. The number of species recorded from each sample by hyphal isolation is given as a general indication of activity of the fungal population. Data on rainfall, soil moisture and date of cultivation of the soil are also given.

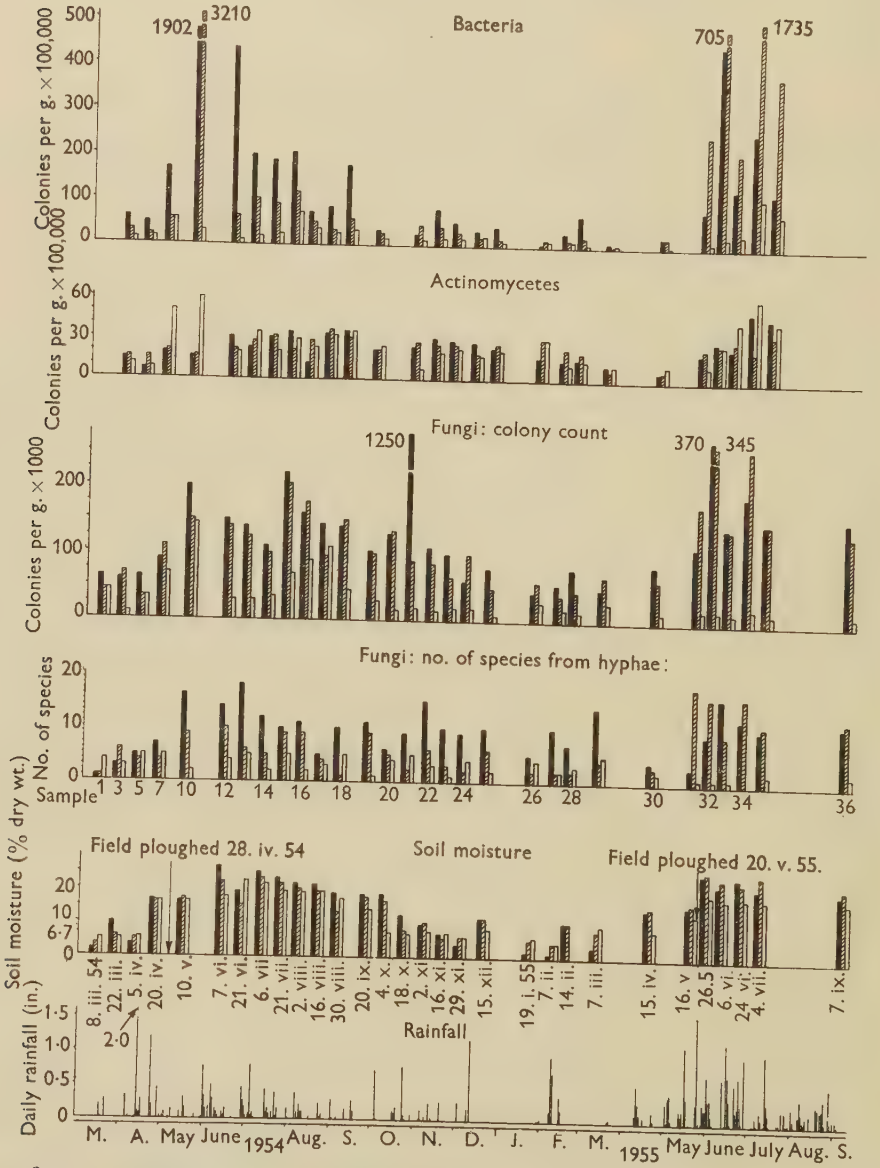
Vertical distribution of the fungal population

The 6 in. sample was originally obtained to study the effect of cultivation on the vertical distribution of fungi in the soil. It was found, however, that most samples obtained from that depth belonged to the subsurface and not to the surface horizon; since there were markedly fewer fungal units in the subsurface than in the surface soil, the effect of cultivation on vertical distribution of fungi could not be followed.

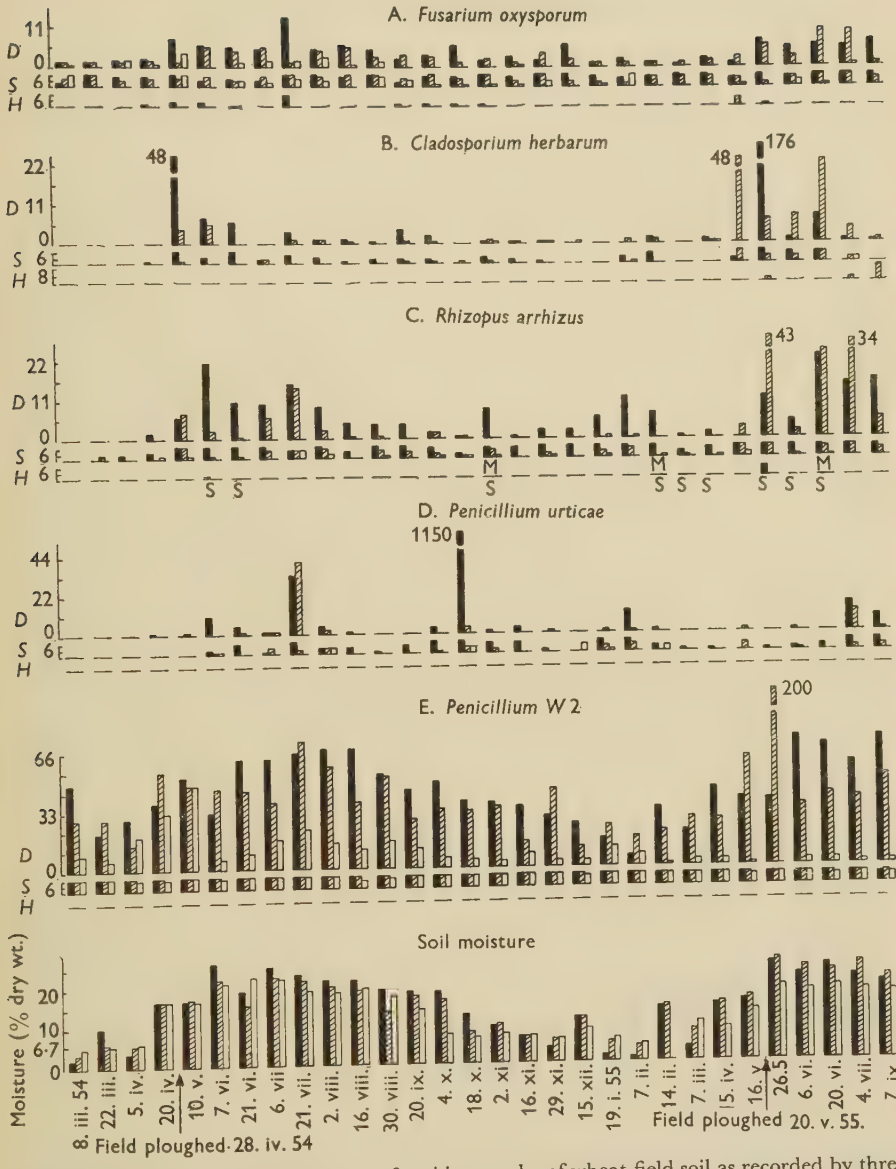
Table 1 and Text-fig. 1 show that significantly fewer fungal units were obtained from the subsurface than from the surface horizon. Counts for the two series of surface samples, at 1 and 3 in. deep, though occasionally showing considerable differences (Text-fig. 1) are not significantly different from one another if the high count for sample 21 is neglected. Since this peak was due entirely to the unusual abundance of *Penicillium urticae* (Text-fig. 2D) in this sample, it is possible to exclude this sample from consideration. Table 1 also shows that fewer species were obtained at 6 in. than from the surface samples by dilution plates, whereas with soil plates a higher number of species was recorded at 6 in. This apparently anomalous result is probably a reflexion of the markedly lower number of fungal units in the subsurface samples; lower numbers of units would give lower numbers of species on dilution plates at 1/5000. On the other hand, the lower number of species on soil plates from surface soil is probably a reflexion of the greater crowding of colonies on these plates. Data from soil and dilution plates show that in general those species which were most commonly encountered in the surface layer were also common in the subsurface soil. This has also been reported by Tresner, Backus & Curtis (1954). Several fungi, however, while common in surface soil were virtually absent from subsurface samples. Table 1 shows that these include *Penicillium urticae*, *Cladosporium herbarum*, *Rhizopus arrhizus* and *Mucor* W13; two other species not included in Table 1 are *Pullularia pullulans* and *Phoma* T29. *Penicillium* W25 and *Arachniotus* W29, however, were recorded more frequently from subsurface than surface samples; but both are slow-growing species, and it is difficult to be certain that they had not been suppressed by faster-growing fungi in plates prepared from surface samples, rather than that they are more abundant in the lower horizon.

Table 1 also shows that fewer fungi were recorded by hyphal isolation from subsurface than from surface samples, both significantly fewer colonies and species being obtained. The full data show that many of the fungi recorded from hyphae from surface samples were absent from subsurface samples and that three sterile species, T58, T9 and T13, were more frequently isolated from subsurface than from surface samples, the fungus T58 often being the only species obtained from hyphae at 6 in.

Text-fig. 1 also shows that bacterial numbers were significantly lower in the subsurface than in the surface samples; with Actinomycetes there was no significant change in number with depth, but a slight increase in number in subsurface samples was recorded.



Text-fig. 1. Occurrence of bacteria, Actinomycetes and fungi in wheat-field soil. Numbers of bacteria, Actinomycetes and fungi per g. air-dry soil, number of species of fungi isolated from hyphae, soil moisture and daily rainfall are recorded. Depth of soil samples is shown as follows: ■, 1 in. deep; ▨, 3 in. deep; □, 6 in. deep.



Text-fig. 2. Occurrence of five different fungi in samples of wheat-field soil as recorded by three different methods. Data for dilution plates (*D*) record number of colonies (in thousands) per g. air-dry soil; for soil plates (*S*) the number of plates (max. 6) on which the fungus occurred is given; and for hyphal isolation (*H*) the number of viable hyphae isolated from each sample is recorded. Depth of soil samples is shown as follows: ■, 1 in. deep; ▨, 3 in. deep; □, 6 in. deep. For *Rhizopus arrhizus*; M = mycelium visible in soil and S = sporangia visible in soil.

Seasonal distribution of the fungal population

Fungal occurrence in the wheat-field soil showed a definite seasonal incidence (Text-fig. 1). During the summer dry period when soil moisture was very low, fungal counts were also low (see also Table 2); with advent of the seasonal rains and increase in soil moisture, there was an increase in the colony count. After cultivation of the field, a further increase in colony count occurred and numbers remained high for some months but gradually fell, especially when the soil dried out. The data for hyphal isolation given in Text-fig. 1 also show a similar picture to that obtained by dilution plates. During the dry period relatively few fungi were isolated from hyphae present in the soil and the percentage growth in agar of the hyphae isolated was low (3–15 %). There was an increase in activity after the seasonal rains commenced, a marked further increase during the period of decomposition of crop residues, thereafter generally falling values except for a possible further slight rise in activity about the time of crop maturity (see also Text-fig. 2). In the summer of 1955, heavy rain in February led to a short burst of activity in the soil before the 'opening' rains in April.

These results show that both fungal hyphae and spores are killed by the low soil moistures of the summer dry period. During the summer the water content of the surface soil may fall to 1·6–2·0 %, whereas the wilting point is 6·7 %; the soil may also be below wilting point to a depth of several feet (Aitchison & Holmes, 1953). These low soil moistures indicate a relative humidity in the soil of 30–50 %.* It is interesting to find that some fungi, notably the sterile species T6, T7, T9 and T58, persisted as hyphae in this dry surface soil for a period of 6–8 weeks: with soil moisture and R.H. so low, it seems unlikely that these hyphae are actively growing. The fact that the colony count is low during the summer means that fungal spores are also killed by the low soil moisture, since the fall in number is much greater than could be attributed to death of hyphae (Table 2). Table 2 also suggests that fungal units in humus particles survive better than spores in dry soil, since a higher percentage of colonies developed from humus particles in samples collected during the dry period than in samples collected when soil moisture was high. Whether this is due to some protection from drying or to a difference in the fungal unit (chlamydospore?) in humus particles is not known. These results would suggest that fungi may also survive over the summer in larger organic fragments in the soil.

Soil fungi may also over-summer either as rhizomorphs or as sclerotia. Several Basidiomycetes have been noted to survive over the summer as rhizomorphs. A study of the sclerotia present in dry soil was made by a nematological cyst flotation technique and showed the presence of six different types of sclerotia in surface soil. Five of these were obtained in culture and include two Basidiomycetes, *Lepiota* T156 and culture T144, *Rhizoctonia* T37, *Aspergillus* W39 and the sterile fungus T52. Several other fungi from the wheat field soil form sclerotia in culture and it is possible that, although not found, sclerotia may also be formed in soil.

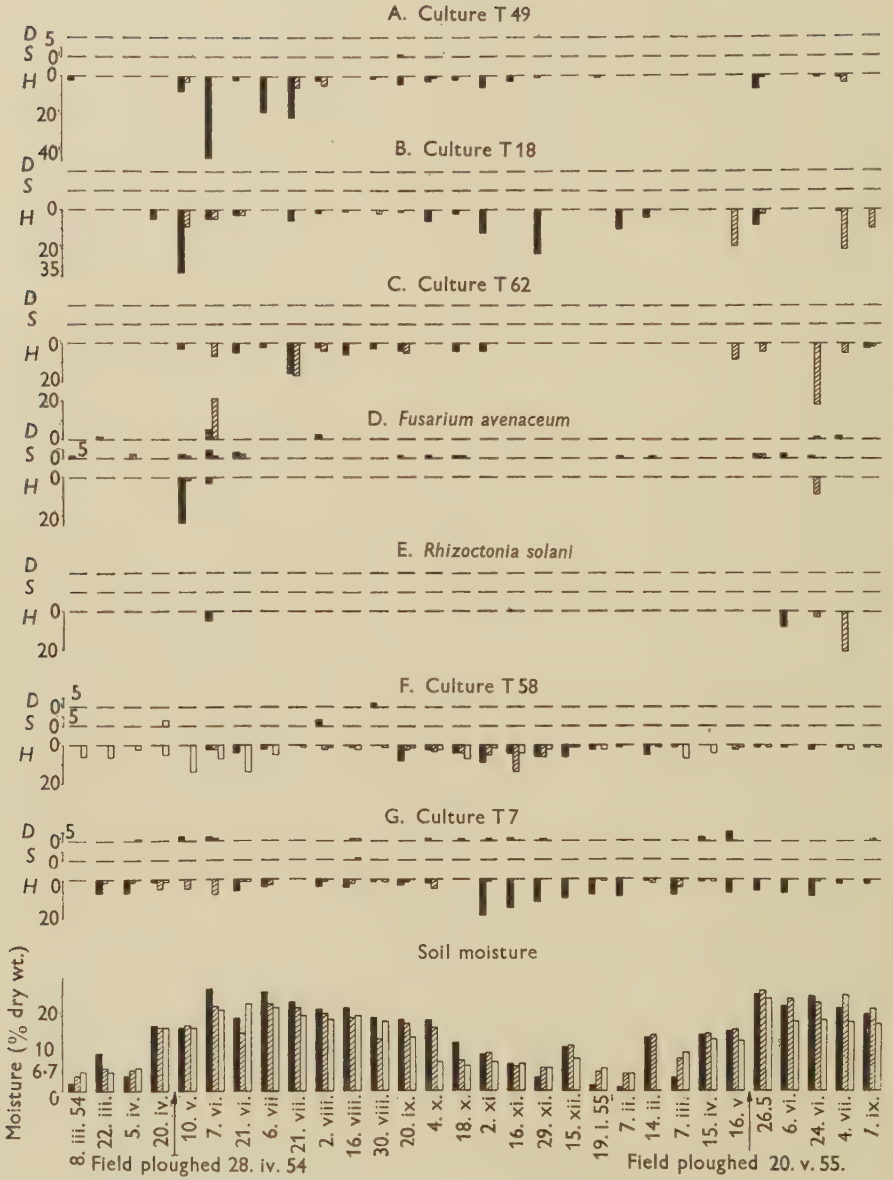
Text-fig. 1 shows that when dry soil becomes moistened after heavy rain

* Data supplied by Dr J. P. Quirk, C.S.I.R.O. Division of Soils.

there is an increase in the fungal count. This increase is probably due to fungal growth and sporulation though it may, in part, be due to liberation of spores from previously formed sporocarps. Pycnidia of several fungi and perithecia of *Chaetomium*, all containing viable spores, have been found in soil during the dry period; such fructifications, however, appear relatively rare. Direct observation suggests that the time interval between germination and sporulation of some fungi in soil may be short: young and mature sporangia of *Rhizopus arrhizus* were found in soil 3 days after the heavy February rain, and sporangia of *Mortierella* and *Mucor* and young fructifications of *Penicillium* have been noted in soil 3–6 days after the start of the seasonal rains.

All data show that cultivation and incorporation of plant residues into the soil is a major factor in the growth of fungi in the wheat-field soil. Dilution plates prepared from samples collected at intervals after cultivation gave increased fungal counts, and hyphal isolation showed an increased amount of mycelium in the soil and also recorded a higher number of species present as hyphae. Viability of hyphae also was higher; up to 75 % instead of an average 23 %, of the hyphae thus isolated grew on agar. Direct observation, as well as the data in Text-fig. 1, showed that the main decomposition took place over a period of 3–4 months after cultivation, although residues were still present in the soil after that period, and some wheat straws may remain in the soil for more than twelve months.

Several fungi were recorded predominantly during the period of decomposition of residues. Data for *Rhizopus arrhizus* and *Cladosporium herbarum* are given in Text-fig. 2 and for *Rhizoctonia solani*, *Fusarium avenaceum* and the sterile fungus T49 in Text-fig. 3. *Rhizopus*, although recorded throughout the year by plating techniques, was found present as hyphae mainly during the early stages of decomposition of residues. In a laboratory study of the decomposition of residues in soil, Martin, Anderson & Goates (1942) found that the higher the percentage of readily decomposable carbohydrate material, sugars, starches and some hemicellulosic material, the greater the predominance of *Mucor* and *Rhizopus*; and Chesters (1949) has commented on the stimulation that the addition of green vegetation to soil gives to mucoraceous fungi in particular. *Cladosporium* was obtained in high numbers shortly after cultivation of the field but was rarely obtained during the rest of the year. *Cladosporium* is a common saprophyte of mature wheat plants and stubble, which suggests that it may enter the soil with wheat residues and sporulate freely after a brief period of growth in and near the residues. In all cases examined *Cladosporium* was present as spores on dilution plates but it was obtained from hyphae three times in 1955. Spores of the fungus appear easily decomposed by other micro-organisms or soon lose their viability in soil, since the number of colonies recorded on dilution plates soon dropped to a low figure. That the spores of fungi may decay rapidly in soil has been shown by several investigators (Chinn, 1953; Park, 1955). Another fungus with a very similar distribution, relatively high numbers during the early stages of decomposition and low numbers during the remainder of the year, was *Pullularia pullulans*. This fungus also occurs on wheat stubble in the field; it was not, however, isolated from hyphae present in the soil. Smit & Wieringa (1953) consider



Text-fig. 3. Occurrence of seven different fungi in samples of wheat-field soil as recorded by three different methods. Data for dilution plates (*D*) and hyphal isolation plates (*H*) record the number of colonies isolated; for soil plates (*S*) the number of plates (max. 6) on which the fungus occurred is given. Depth of soil samples is shown as follows: ■, 1 in. deep; ▨, 3 in. deep; □, 6 in. deep.

that *Pullularia pullulans* is the chief pectin-splitting organism in the early decomposition of litter. Another fungus that may enter the soil with wheat straw is *Dinemasporium graminum*. It was isolated from soil during the period of decomposition in 1955 and pycnidia of the fungus have often been found on buried wheat straw in the soil. Both *Rhizoctonia solani* and the sterile fungus T49 appeared most abundantly during the period of decomposition, but whether these fungi play any part in decomposition is not known. *Fusarium avenaceum* was also recorded from hyphae only during this period and supplementary studies suggest that it may be important in the decomposition of leaf tissue in the soil.

Although the data show that fungal activity in the soil was highest during the decomposition of residues, little information has been gained on the fungal flora most active in decomposition. Some studies of decaying leaf tissue suggest that some of the fungi actively degrading the tissue are scarcely recorded by the hyphal isolation method. Further studies are being undertaken on the decomposition of wheat residues in soil.

Text-fig. 1 also shows that the bacterial count reached high figures during decomposition of crop residues. These and supplementary samples showed, as has been reported by Humfeld & Smith (1932) and Dawson, Dawson & McCalla (1948), that very high numbers of bacteria were limited almost entirely to the region of the soil in which the residues occurred. In contrast to bacteria and fungi, Actinomycetes showed comparatively little increase in number after cultivation and incorporation of residues in the soil.

It is interesting to note that all data (Text-figs. 1–3) show that microbial activity (and residues) occurred in different zones in the soil in the two years. In 1954 activity and residues occurred predominantly in the 1 in. samples, whereas in 1955 they occurred predominantly in the 3 in. samples. This was due to different cultivations in the two years: in 1954 the soil was first cultivated with a 'Ferguson' tiller which broke up the surface soil to a depth of 2–3 in., and later the field was ploughed: in 1955 the field was merely ploughed. Ploughing alone turned in most of the residues to a depth of 3–5 in., but cultivation and later ploughing distributed many residues nearer the surface of the soil.

Occurrence and activity of specific fungi in soil

Over 210 species of fungi have been isolated from the wheat-field soil, a much higher number than has been previously recorded from one soil. Much of the increase is due to the use of the hyphal isolation method, which isolated many species not recorded by dilution and soil plates. It should be emphasized, however, that this list is not a complete enumeration of all fungi that occur either actively or passively in Urrbrae loam. Unpublished data show that this soil under pasture contains fungi not yet recorded from the wheat-field; it is also known that Basidiomycetes other than those recorded here occur in the wheat-field. Thus the total number of species that may occur in a soil becomes rather staggering, particularly if all species have a role in soil transformations.

Data on the occurrence and activity of individual fungi are considered

in this section. As previously discussed, data on activity are considered to be limited since certain specific fungal habitats, grosser organic fragments and plant roots have not been examined; also direct observation suggests that some fungi are active in restricted areas or for short periods of time and are not likely to be recorded adequately by a sampling procedure of the type used in this investigation.

Phycomycetes were represented in the wheat-field soil by twenty-three species of twelve genera. Four species belonged to the Oomycetes, the remainder were Zygomycetes. *Pythium* was rarely obtained during the summer dry period but was present in low numbers in most samples collected during the remainder of the year when soil moisture was high. Several workers (Chesters, 1949; Warcup, 1951) have recorded that *Pythium* shows a seasonal distribution, being more regularly isolated when soil moisture is relatively high. *Pythium* occurred at all three depths sampled, but was most abundant in the 1 in. samples; it was obtained from soil plates more frequently than from dilution plates, and was also isolated from hyphae in samples 10 and 11 in May 1954, about one month after cultivation of the field. In both cases it was found that, although extensive branched mycelium was present, growth in agar occurred only from hyphal tips and young oogonia, the remainder of the hyphal mass appearing non-viable. This suggests that the life of an individual *Pythium* hypha in soil may be of short duration. Somewhat surprisingly in 1955, a wetter year than 1954, *Pythium* was not recorded from hyphae. The identity of the Pythia obtained was not investigated, but Kerling (1947) has recorded *P. debaryanum*, *P. vexans* and *P. polymorphon* from red-brown earth at the Waite Institute. The chytrid *Rhizophydium sphaerotheca* was isolated once from the surface of an unidentified phycomycetous hypha on a hyphal isolation plate. *Aphanomyces* was also obtained once, being isolated from hyphae present in the soil after the heavy summer rain in February 1955.

Of the Zygomycetes, *Rhizopus arrhizus*, *Mucor* W13, *Absidia butleri* and *Mortierella* spp. were relatively abundant; the remaining species were rarely obtained and little is known of their role in soil. The data for *R. arrhizus* (Text-fig. 2C) has already been discussed; the data for *Mucor* are similar, low counts during the summer, higher counts during decomposition of residues, and the fungus has been seen fruiting in worm tunnels and on the bases of maturing wheat plants, but so far it has not been obtained by hyphal isolation. *A. butleri* differed from most other Zygomycetes in that it was recorded more frequently from the 3 and 6 in. than from the 1 in. samples. Zygotes of this species were occasionally present in soil plates; the fungus was not recorded from hyphae. *Mortierella* spp. were isolated more frequently from soil than from dilution plates. Lower fungal counts were recorded during the summer than during the winter, and these fungi were also recorded from hyphae on several occasions. Observations on the origins of colonies on dilution plates showed that *Mortierella* developed from sporangiospores, stylospores or hyphal chlamydospores. *Mortierella* has also been found fruiting in worm tunnels after the opening rains.

Besides these fungi, phycomycetous hyphae bearing vesicles were common in the wheat-field soil and in some samples represented a considerable proportion of the hyphae present. Several of the vesicle-bearing

hyphae appear similar to the type A spores obtained from Illinois soil by Gerdemann (1955). Gerdemann records Type A spores as borne in irregular groups and not exceeding 200μ in diameter. Hyphae are attached directly to the spore without a bulbous swelling and the spores are not separated from the hyphae by a cross-wall. He considers that these spores are similar to those discussed by Butler (1939) as possible chlamydospores of the Endogonaceae. The type B and C spores of Gerdemann were not seen in this study but one or two other types of vesicle were noted. None of these vesicle-bearing fungi was obtained in culture, although a few hyphae of one type showed slight growth on agar. The absence of these fungi from the fungal record is a major omission from the fungi isolated from this soil.

Ascomycetes were represented by eleven genera and thirteen species. Apart from *Arachniotus* W29 and *Thielavia* W16, most were rare or of sporadic occurrence. *Arachniotus* was most frequently isolated from the lower soil samples; *Thielavia* occurred in relatively low numbers from most samples obtained, and did not show a drop in number during the summer dry period. *Thielavia* was obtained once by hyphal isolation, occurring as mycelium inside a dead hypha of *Rhizopus*. It is possible, but unlikely, that it was acting as a parasite; it is more probable that it was growing as a saprophyte inside a dead hypha. Several different fungi have been found inside other hyphae, the most common being the sterile fungi T7 and T58. Ascocarps of *Thielavia* have also been found on wheat straw in the soil. The most interesting Ascomycete isolated was *Ophiobolus graminis*, which is not usually reported from soil. *O. graminis* was isolated from four samples, twice in 1954 and twice in 1955; the first two samples were not noted to be associated with disease in the crop, but the latter two were obtained from an area showing seedling disease. All isolates obtained were found to be pathogenic to wheat seedlings in pot tests; perithecia were obtained on wheat seedlings in pathogenicity tests and in culture on sterilized soil and chaff.

Ten Basidiomycetes were isolated from the wheat-field soil, eight by hyphal isolation and two from sclerotia. Eight of the ten possess clamp connexions and have not fruited in culture; the remaining two include *Pellicularia filamentosa* (*Rhizoctonia solani*), and a species that has formed fructifications in culture on a straw and soil medium and that appears to be a *Lepiota*. The data for *R. solani* are shown in Text-fig. 3E. The pathogenicity of all isolates was tested against wheat, tomato, radish and lettuce seedlings. All isolates caused damping-off of radish seedlings but not of any other host tested, and therefore belong to the crucifer strain of *R. solani* (Storey, 1941).

As is typical in soil isolations, most of the fungi isolated must be placed in the Fungi Imperfecti. Some species were abundant, others were isolated only once. Prominent genera were *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Phoma*. As previously mentioned, an unusual feature of the investigation is the large number of fungi, about eighty, that have remained sterile in culture and most of which were isolated by the hyphal isolation method. Nine species of *Aspergillus* were obtained, but only two species, *A. terreus* and *A. flavus*, were at all common. Both species

were obtained in low numbers throughout the year; no *Aspergillus* was obtained by hyphal isolation. Conidial heads of *A. flavus* and *A. terreus* were both found on a dead beetle in the soil, and *A. terreus* was occasionally seen fruiting on the base of wheat stubble. Four species of *Fusarium* were obtained, *F. oxysporum* being by far the most abundant. Gordon (1954) also found this species the most abundant *Fusarium* in wheat plots in Canada. Data on *F. oxysporum* are given in Text-fig. 2A and show that this fungus was present in all samples obtained, but was usually isolated in lower numbers from the subsurface horizon. Higher counts of *F. oxysporum* were obtained during the wet than during the dry season. The fungus was also isolated from ten samples by the hyphal isolation method, being found both before and after the main period of decomposition, suggesting that its activity is not dependent on the incorporation of residues into the soil. In examination of dilution plates, *Fusarium* colonies were found developing from humus particles or chlamydospores and only rarely from microconidia, while macroconidia were not recorded. Ram (1952) has reported that chlamydospore production by *F. solani* is stimulated by two soil bacteria. Pathogenicity tests showed that isolates of *F. avenaceum*, alone of the *Fusarium* isolates from the wheat-field, were weakly pathogenic to wheat seedlings; the importance of this species in the field is not known.

Sixteen species of *Penicillium* were isolated from the wheat-field soil; some species, as shown in Table 1, were present in high numbers, other species were seldom found. *Penicillium* W2 and *P. urticae* had higher counts than any other fungi isolated, but, as shown in Text-fig. 2D, E, they had very different distributions in the soil, *Penicillium* W2 being constantly present in relatively high numbers and *P. urticae* being very erratic. Text-fig. 2D shows that the counts for *P. urticae* include the very high figure recorded from sample 21. Examination of dilution plates of this sample showed no colonies developing from hyphae, so that this peak was due to an accumulation of spores in the soil. Direct observation indicated that such an accumulation of spores may occur with this species, since on one occasion masses of spores of *P. urticae* were seen in the soil spreading over an area of 2 × 1 cm.; the nature of the substrate on which the fungus was sporing so profusely is not known. Little information on the activity of *Penicillium* in soil has been obtained in this study, but it seems probable that the conditions governing the growth of *Penicillium* W2, with a comparatively uniform distribution of spores throughout the soil, must be different from those operating for *P. urticae*, with high numbers of spores occurring in restricted loci. Though hyphal isolation may be selective against *Penicillium*, it is of interest to record that *Penicillium* hyphae have not been found on any of the dilution plates examined for origins of fungal colonies. Much further information is needed before the activity of *Penicillium* in the wheat-field can be defined.

Seven species of *Phoma* were recorded, mainly by dilution and soil plates, though occasionally from hyphae. Most showed sporadic occurrence and little information is available on their activity in soil. *Trichoderma viride* is another fungus that was recorded more frequently from soil than from dilution plates. It occurred in low numbers in many samples, and was less frequently isolated during the summer than during the remainder of the

year. It was obtained once by hyphal isolation, and has also been seen fruiting on buried straw in the soil.

A large number of sterile forms (Pl. 10, fig. 3) was obtained in this study. Some have white or pink hyphae, but most have brown or black; some are fast-growing, but many are slow-growing, some being extremely slow-growing on all substrates tested. The data for five species, T7, T18, T49, T58 and T62 are given in Text-fig. 3. In all cases these fungi were rare or absent from dilution and soil plates. T49 and T62 are species that were isolated most frequently during the decomposition of residues, and neither was recorded during the summer dry period. The species T7 and T58, which are slow-growing forms with black hyphae, were found throughout the year and showed little or no increase in frequency during the decomposition of residues. T58 was the most abundant fungus isolated from subsurface soil and appears widespread, since it was obtained from all samples collected during the investigation. The fungus T18 was active during decomposition, but was also found at other periods of the year. It was noted that the hyphae of T18 were very variable in soil, sometimes being large, 8–15 μ in diameter, whereas in agar its growth was regular with a hyphal diameter of 5–7 μ.

Discussion

The investigations recorded in this paper on the ecology of fungi in a wheat-field have been of a general nature and, of necessity, many interesting questions remain untouched. The data gained, however, show that the method of investigation used profoundly influences the picture obtained of the fungal flora of the soil, both with regard to the occurrence and the activity of its members. Hyphal isolation, besides giving information on activity of fungi in soil, has allowed many fungi to be isolated that were not recorded by plating techniques, and has indicated the presence of others, not yet isolated from soil. Direct observation of soil has also provided information not obtained by any other method used, indicating that many methods are necessary to gain an understanding of a soil population.

The data obtained show that there is a marked seasonal distribution of fungi in the wheat-field soil. Both hyphae and spores have been shown to be killed during the summer dry period. This conclusion is contrary to those of Erdman (1928) and Singh (1937) but, in this study, a greater number of samples has been obtained than has been usual; also, and probably more important, the seasonal changes in Adelaide, particularly in relation to soil moisture, are more severe than those experienced where other studies have been carried out. Some fungi have been found to survive in soil as hyphae over the dry period and this fact raises several questions. It suggests that the occurrence of hyphae of a fungus in soil may not necessarily be an indication of current activity of that fungus, unless some of the fungi recorded in this investigation can grow at very low soil moistures. Another question is how long can such hyphae survive in soil? It is generally considered that hyphae are short-lived in soil (Waksman, 1927; Starkey, 1938; Russell, 1950), through being attacked by other micro-organisms. Certainly in dry soil some fungi can survive as hyphae

for long periods, hyphae having been isolated from dry soil stored in the laboratory for over 12 months. There is less information on their survival in moist soil, where they would be subject to microbial attack, but what little evidence is available suggests that they may be relatively long-lived. On the other hand, many of the fungi isolated from the wheat-field were shown to have short-lived hyphae in soil.

All methods used here showed that cultivation of the soil and incorporation of residues are major factors in the activity of fungi in the wheat-field soil. Many fungi were isolated only during the period of decomposition of residues; others occurred most abundantly at that time, but the data do not show which fungi are most active in decomposition. Much of the data on activity of fungi in soil was obtained by hyphal isolation; this is, however, a general method and gives little information on fungi that may be present in residues or other organic fragments in soil. The limited data obtained show, as has been suggested by Chesters (1949) and Garrett (1955), that these specialized habitats are important and must be studied to obtain a full understanding of the occurrence and activity of fungi in soil. Plant roots are another special habitat not specifically studied in this investigation.

Over 210 fungi were obtained from the wheat-field soil during this investigation and supplementary studies suggest that this number does not represent a complete list of the fungi present, either actively or passively, in this soil. With such large numbers, the problem of investigating their activity becomes great and the role of the majority obtained is not known. An interesting problem is what the fungi that occur but once or twice in an investigation represent. Chesters (1949) has suggested that the occasional species of such lists may be derived from spores of Fungi Imperfecti which are living in organic debris, or from spores which have been carried down from above. It is possible that some may represent spores from the air or from organic debris on the surface of the soil, and others may represent spores of fungi living in organic debris in soil, but as yet no information is available to differentiate fungi in these different classes. The data obtained in this study, in which fungi found but occasionally on soil or dilution plates were found to be common as hyphae in soil, suggest that we cannot lightly dismiss rare fungi in our investigations as being of little importance in soil.

I wish to express my thanks to Dr C. G. Hansford for his continued interest in this work and for assistance in identification of fungi; to Dr W. L. Gordon for identification of *Fusarium*; to Dr C. W. Hesseltine for identification of *Helicostylum*; to Dr M. B. Ellis for identification of *Periconia*; to Prof. F. K. Sparrow for identification of *Rhizophydium* and to Mr K. P. Phillips for the photographs.

Appendix

List of species isolated

Phycomycetes

Oomycetes

m*	*Rhizophydium sphaerotheca* Zopf
m	*Aphanomyces* T 101
dsm	*Pythium* spp.

Zygomycetes

ds	*Absidia butleri* Lendner
d	*A. ?ramosa* (Lindt) Lendner
ds	*Actinomucor repens* Schostakowitsch
s	*Circinella muscae* (Sorok.) Berl. & de Toni
d	*C. umbellata* van Tiegh. & Le Monn.
ds	*Cunninghamella* W 22
d	*Helicostylum pyriforme* Bain.
dsm	*Mortierella*: 5 spp.
ds	*Mucor* W 13
s	*Mucor* spp.
dsm	*Rhizopus arrhizus* Fischer
ds	*R. stolonifer* (Fr.) Lind.
d	*Syncephalis* W 159

Ascomycetes

Plectomycetes

ds	*Arachniotus* W 29
ds	*Aspergillus* SA 15
ds	*A. fischeri* Wehm.
dm	*Emericellopsis mirabilis* (Malan) Solk
s	*?Myxotrichum* W 84
dsm	*Thielavia* W 16
s	Culture W 40

Pyrenomycetes

ds	*Chaetomium funicola* Cooke
ds	*Chaetomium* W 70
s	*Melanospora* W 23
s	*Neocosmospora vasinfecta* E. F. Smith
m	*Ophiobolus graminis* (Sacc.) Sacc.
d	*Pleospora* W 115

Basidiomycetes

+	*?Lepiota* T 156
m	*Pellicularia filamentosa* (Pat.) Rogers
m	Cultures T 14, T 36, T 46, T 80, T 146, T 152 and T 154
+	Culture T 144

Fungi imperfecti

ds	*Acremonium* W 59
dsm	*Alternaria tenuis* auct.
ds	*Alternaria* W 54
m	*Alternaria* T 118
d	*Alternaria* W 131
dsm	*Ascochyta* T 141
ds	*Aspergillus flavipes* (Bain. & Sart.) Thom
ds	*A. flavus* Link
ds	*A. fumigatus* Fres.
ds	*A. niger* van Tiegh.
ds	*A. terreus* Thom.
ds	*Aspergillus*: 2 spp.
dsm	*Cladosporium herbarum* Fr.
ds	*Cephalosporium*: 3 spp.
dsm	*Cylindrocarpon ?ehrenbergi* Wr.
m	*Dinemasporium graminum* (Lib.) Lév.
d	*Epicoccum purpurascens* Ehrenb.
m	*Fusarium acuminatum* Ell. & Ev.
dsm	*F. avenaceum* (Fr.) Sacc.
dsm	*F. oxysporum* sensu Snyder & Hansen
ds	*F. solani* sensu Snyder & Hansen
ds	*Gliocladium fimbriatum* (series)
ds	*G. roseum* (series)
ds	*Gliomastix convoluta* (Harz) Mason
ds	*Haplosporella* W 86
d	*Helicodendron* W 85
m	*Helicosporium* T 99
d	*Helminthosporium* W 100
ds	*Humicola grisea* Traaen
ds	*Humicola* W 61
s	*Myrothecium* W 164
ds	*?Naemospora* W 57
d	*Oospora* W 129
ds	*Papularia sphaerosperma* (Pers.) von Höhnel
ds	*Periconia macrospinosa* Lefebvre & A. G. Johnson
ds	*Penicillium lilacinum* Thom
ds	*P. melinii* Thom
ds	*P. purpurogenum* Stoll
ds	*P. urticae* Bain.
ds	*Penicillium* W 2
ds	*Penicillium* W 3
ds	*Penicillium*: 10 spp.
ds	*Pestalotia* W 67
m	*Phialophora* T 100
dsm	*Phoma* T 29
s	*Phoma* W 73
d	*Phoma*: 5 spp.
ds	*Pullularia pullulans* (de Bary & Low) Berkh.
ds	*?Scopulariopsis* W 21
ds	*Stachybotrys atra* Fr.
d	*Stemphylium botryosum* Wallr.
ds	*Stemphylium*; 3 spp.
dsm	*Trichoderma viride* Fr.
d	Cultures W 69, W 93, W 112, W 118, W 120, W 147, W 167 and W 179

ds Cultures W82, W88, W103, W151 and W190
dm Cultures T33 and T61
m Culture T111

Sterile cultures

m *Rhizoctonia*: 4 spp.
d Cultures W94, W96, W98, W113, W114, W126, W135, W150, W152, W160, W161, W163, W175 and W177
ds Cultures W9, W56, W79, W83, W108 and W154
s Cultures W89, W111, W130 and W132
dsm Cultures T6, T7, T42, T48, T58, T67, T90 and T95
dm Cultures T9, T108 and T134
sm Cultures T13, T41, T49, T52 and T60
m Cultures T4, T8, T11, T18, T24, T25, T30, T31, T39, T40, T45, T47, T50, T53, T54, T55, T59, T62, T68, T69, T70, T75, T87, T88, T89, T97, T103, T104, T109, T117, T119, T112, T126, T128, T130, T136, T139, T140 and T145

* Method of isolation; m = hyphal isolation; d = dilution plate method; s = soil plate method; + = isolated from sclerotia present in soil.

REFERENCES

Aitchison, G. D. & Holmes, J. W. (1953). Seasonal changes of soil moisture in a red-brown earth and a black earth in southern Australia. *Aust. J. appl. Sci.* **4**, 260–273.

Angell, H. R. (1954). Partial segregation of bacteria and isolation of *Pythium* from the coarser soil fractions. *Aust. J. agric. Res.* **5**, 702–705.

Butler, E. J. (1939). The occurrences and systematic position of the vesicular-arbuscular type of mycorrhizal fungi. *Trans. Brit. mycol. Soc.* **22**, 274–301.

Chesters, C. G. C. (1949). Concerning fungi inhabiting soil. *Trans. Brit. mycol. Soc.* **32**, 197–216.

Chinn, S. H. F. (1953). A slide technique for the study of fungi and actinomycetes in soil with special reference to *Helminthosporium sativum*. *Canad. J. Bot.* **31**, 718–724.

Dawson, R. C., Dawson, V. T. & McCalla, T. M. (1948). Distribution of micro-organisms as affected by plowing and subtilling crop residues. *Res. Bull. Neb. agric. Exp. Sta.* no. 155, 1–26.

Erdman, L. W. (1928). The numbers of micro-organisms in Carrington loam as influenced by different soil treatments. *Res. Bull. Ia. agric. Exp. Sta.* no. 109.

Garrett, S. D. (1955). Microbial ecology of the soil. *Trans. Brit. mycol. Soc.* **38**, 1–9.

Gerdemann, J. W. (1955). Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. *Mycologia*, **47**, 619–632.

Gordon, W. L. (1954). The occurrence of *Fusarium* species in Canada. IV. Taxonomy and prevalence of *Fusarium* species in the soil of cereal plots. *Canad. J. Bot.* **32**, 622–629.

Harley, J. L. & Waid, J. S. (1955). A method of studying active mycelia on living roots and other surfaces in the soil. *Trans. Brit. mycol. Soc.* **38**, 104–118.

Humfeld, H. & Smith, N. R. (1932). The decomposition of vetch green manure in relation to the surrounding soil. *J. agric. Res.* **44**, 113–120.

Kerling, L. C. P. (1947). *Pythium debaryanum* and related species in South Australia. *Trans. roy. Soc. S. Aust.* **71**, 253–258.

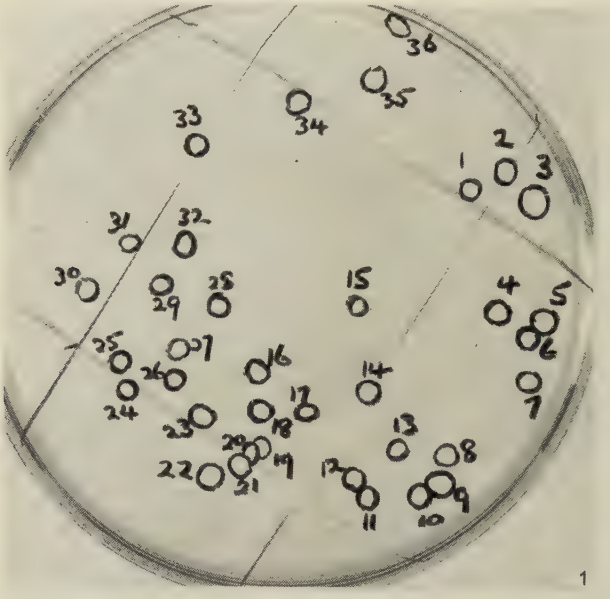
Kubiena, W. S. (1938). *Micropedology*. Iowa: Collegiate Press.

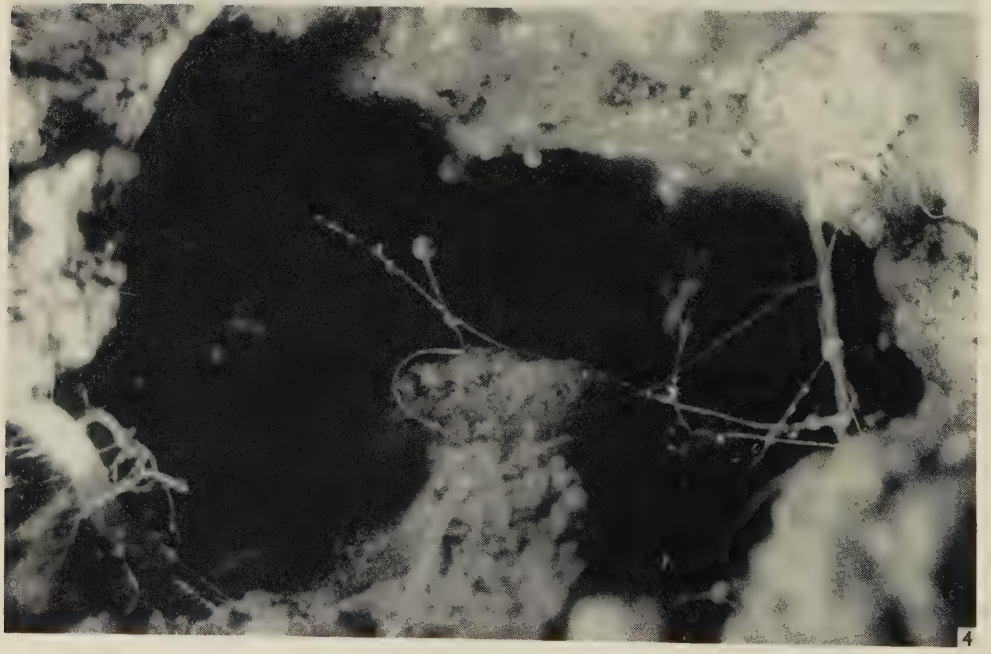
Kubiena, W. S. & Renn, C. E. (1935). Micropedological studies of the influence of different organic compounds upon the microflora of the soil. *Zbl. Bakt.*, (2 Abt.), **91**, 267–292.

Langeron, M. (1945). *Précis de mycologie*. Paris: Masson.

McLennan, E. I. & Ducker, S. C. (1954). The ecology of the soil fungi of an Australian heathland. *Aust. J. Bot.* **2**, 220–245.

Martin, T. L., Anderson, D. A. & Goates, R. (1942). Influence of the chemical composition of organic matter on the development of mold flora in soil. *Soil. Sci.* **54**, 297–302.





(*Facing p.* 259)

Park, D. (1955). Experimental studies on the ecology of fungi in soil. *Trans. Brit. mycol. Soc.* **38**, 130–142.
Piper, C. S. (1938). The red-brown earths of South Australia. *Trans. roy. Soc. S. Aust.* **62**, 53–100.
Ram, C. S. V. (1952). Soil bacteria and chlamydospore formation in *Fusarium solani*. *Nature, Lond.*, **170**, 889.
Raper, J. R. (1940). Sexuality in *Achlya ambisexualis*. *Mycologia*, **32**, 710–727.
Raper, K. B. & Thom, C. (1949). *A Manual of the Penicillia*. London: Baillière, Tindall and Cox.
Russell, E. J. (1950). *Soil conditions and plant growth*. London: Longmans, Green and Co.
Singh, J. (1937). Soil fungi and actinomycetes in relation to manurial treatment, season and crop. *Ann. appl. Biol.* **24**, 154–168.
Smit, J. & Wieringa, K. T. (1953). Microbiological decomposition of litter. *Nature, Lond.*, **171**, 794–5.
Starkey, R. L. (1938). Some influences of the development of higher plants upon the microorganisms in the soil. VI. Microscopic examination of the rhizosphere. *Soil Sci.* **45**, 207–250.
Storey, I. F. (1941). A comparative study of strains of *Rhizoctonia solani* (Kühn) with special reference to their parasitism. *Ann. appl. Biol.* **28**, 219–228.
Thornton, R. H. (1956). *Rhizoctonia* in natural grassland soils. *Nature, Lond.*, **177**, 230–231.
Thom, C. (1927). Present and future studies of soil fungi. *Proc. 1st. Congr. Soil Sci.* **3**, 39–47.
Timonin, M. I. (1940). Interaction of higher plants and soil micro-organisms. II. *Canad. J. Res.* C, **18**, 444–455.
Tresner, H. D., Backus, M. P. & Curtis, J. T. (1954). Soil fungi in relation to the hardwood forest continuum in southern Wisconsin. *Mycologia*, **46**, 314–333.
Waksman, S. A. (1927). *Principles of soil microbiology*. London: Baillière, Tindall and Cox.
Waksman, S. A. (1944). Three decades with soil fungi. *Soil Sci.* **58**, 89–114.
Warcup, J. H. (1950). The soil-plate method for isolation of fungi from soil. *Nature, Lond.*, **166**, 117.
Warcup, J. H. (1951). The ecology of soil fungi. *Trans. Brit. mycol. Soc.* **34**, 376–399.
Warcup, J. H. (1952). Effect of partial sterilization by formalin on damping-off of Sitka spruce. *Trans. Brit. mycol. Soc.* **35**, 248–262.
Warcup, J. H. (1955*a*). Isolation of fungi from hyphae present in soil. *Nature, Lond.*, **175**, 953.
Warcup, J. H. (1955*b*). On the origin of colonies of fungi developing on soil dilution plates. *Trans. Brit. mycol. Soc.* **38**, 298–301.

EXPLANATION OF PLATE 10

Fig. 1. Hyphal isolation plate with hyphae located and ringed on the reverse of the plate.
Fig. 2. Hypha of *Rhizoctonia* T 115 isolated from soil, showing new growth in agar. × 200.
Fig. 3. Hypha of culture T 53 isolated from soil, showing new growth in agar. × 200.
Fig. 4. *Rhizopus arrhizus* sporing in a worm tunnel in the wheat-field soil. × 10.

(*Accepted for publication* 26 *April* 1956)

Trans. Brit. mycol. Soc. **40** (2), 260–262 (1957).

A *CERCOSPORA* LEAF SPOT OF RAMIE IN ASSAM

By S. CHOWDHURY
Mycologist, Assam, Jorhat, India

(With 1 Text-figure)

A *Cercospora* leaf spot of ramie (*Boehmeria nivea*) is reported from Assam. The disease produces dark brown to nearly black spots on the leaves, but causes only slight damage.

The causal fungus has been identified as *C. boehmeriae* Peck and its pathogenicity has been established by inoculation. The morphology of the fungus, which grew well in a number of culture media but failed to produce spores under varied conditions of temperature, hydrogen-ion concentration, light and humidity, is described. The disease can be controlled by spraying with copper fungicides.

Introduction

Ramie (*Boehmeria nivea* Hook. et Arn.) is grown in parts of Assam for its fibre, which is used mainly for the manufacture of fishing nets. In October 1953, a leaf spot disease of ramie due to a species of *Cercospora* was noticed for the first time at Barbheta, Assam. Since then it has been noticed every year at Barbheta and surveys have shown it to be common and widespread in nearly all the ramie-growing tracts of the State.

The damage caused by the disease is usually slight. Affected plants are not killed outright. When the spotting is very severe, and if the plant is young, the growth is retarded.

Symptoms

The disease appears as circular to angular spots on the upper surface of the leaf. Sometimes the spots which are 1–8 mm. in diameter and dark brown to nearly black in colour are limited by the leaf veins. The centre of older spots, however, turns paler and becomes greyish brown; adjacent spots may coalesce. No symptoms have been seen on the stem; the petioles, however, often show brown elongated spots.

The disease has been observed practically throughout the year, but the severest infection occurs from the latter part of September to early January. The development of the disease is favoured by moist and cool weather.

Infection is usually more severe on the lower than on the upper leaves. In severe infection more than 60 % of the leaves are affected and 35–60 % of the leaf area, on an average, destroyed by spotting. Leaves severely spotted turn yellow and fall prematurely.

Morphology of the parasite on the host

The *mycelium* is both inter- and intracellular. The young hyphae are hyaline and sparsely septate, while the older ones are brown to dark brown, more frequently septate, and constricted at the septa. The hyphae

grow into the air spaces under the stomata and form stromatic masses from which conidiophores emerge through the stomata. The *conidiophores* (Fig. 1 *a*) arise in tufts. They are short, pale olivaceous brown, sparingly septate, unbranched, rarely geniculate, sometimes slightly wavy, 15–95 × 3–4 μ. Conidial scars are absent or very indistinct. The *conidia* (Fig. 1 *b*) are long, septate, cylindrical, clavate to subclavate, tapering towards the end, usually curved and subhyaline, septa mostly not prominent, 40–120 × 3–4 μ.

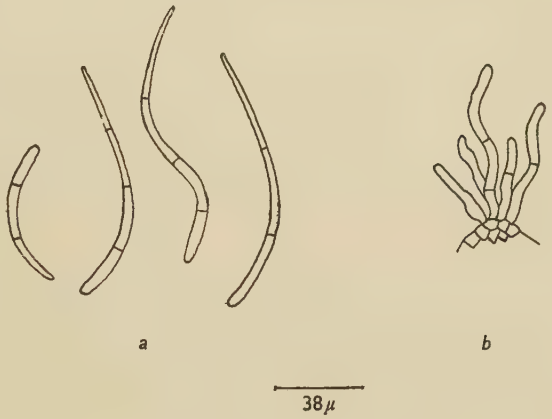


Fig. 1. *Cercospora boehmeriae* Peck. *a*, Conidia; *b*, conidiophores.

Identification of the parasite

So far, two species of *Cercospora* have been recorded on species of *Boehmeria* (Muller & Chupp, 1936; Chupp, 1953). They are: *C. boehmeriae* Peck and *C. krugiana* Muller & Chupp. The reported spore measurements for these two fungi and those of *Cercospora* on ramie from Barbheta are given in

Table 1. *Comparative spore measurement data of the species of* Cercospora *on* Boehmeria

	Conidia			Conidiophores		
Fungi	Length (μ)	Width (μ)	Colour	Length (μ)	Width (μ)	Colour
C. boehmeriae	40–125	2·5–4	Subhyaline to pale olivaceous	15–60	2·5–4	Pale olivaceous brown
C. krugiana	40–110	2·5–4	Hyaline	30–110	4·5–6	Grey
Cercospora sp. from Assam	40–120	3–4	Subhyaline	15–95	3–4	Pale olivaceous brown

Table 1, from which it is clear that the *Cercospora* occurring on ramie is similar to *C. boehmeriae* with which it is identified. This fungus has been reported from Uganda (Hansford, 1943) and from Formosa (Yamamoto, 1934). This, however, appears to be the first report of this fungus in India.

Growth in culture and pathogenicity

A large number of single spore isolations were made from the leaves and the petioles. All were identical.

The fungus did not sporulate when grown on a range of media, and so for pathogenicity tests inoculum was prepared by macerating 9- to 12-day-old cultures on oatmeal agar in water by means of a mechanical blender according to the method suggested by Andrus (1941). The inoculum so prepared was sprayed with an atomizer on the leaves of the plants grown in sterilized soil in cement pots and in Erlenmeyer flasks in Knop's solution. Inoculation experiments were also carried out by placing bits of culture of the fungus on the leaves, petioles and stems. The inoculum was kept in place and moist by covering it with small piece of sterile absorbent cotton soaked in sterile water. All inoculated plants were kept inside glass chambers and sprayed occasionally with sterile water.

After 5 days all the plants were removed from the glass chambers and kept out of doors.

Infection was obtained whenever the leaf or the petiole was inoculated. Stems could not be infected. The first symptoms appeared 12–14 days after inoculation by spraying and after 7–8 days when bits of culture were used.

The symptoms produced by inoculation were typical of the disease as it occurs naturally. The fungus sporulated on the inoculated plants. It was re-isolated and it produced the disease when re-inoculated into healthy plants.

Control

In October 1954, a planting of ramie containing 77 diseased plants in a nursery at Barbheta was divided into four sections and treated as follows: (1) control, not treated; (2) 20 plants, sprayed with 4:4:50 Bordeaux mixture; (3) 20 plants, sprayed with Yellow Cuprocide at the rate of 3 lb./100 gal. water; (4) 20 plants, sprayed with Perenox at the rate of 3 lb./100 gal. water.

Spray applications were given on 15 September, 15 October, 15 November and 15 December. All the treatments gave practically complete control.

The author's thanks are due to Dr S. P. Wiltshire, Director, Commonwealth Mycological Institute, Kew, for furnishing literature.

References

Andrus, C. F. (1941). Preparation of inoculum with a mechanical liquefier. *Phytopathology*, **31**, 566–567.

Chupp, C. (1953). *A Monograph of the genus* Cercospora, pp. 582–583. Ithaca, New York.

Hansford, C. G. (1943). Contributions towards the fungus flora of Uganda. V. Fungi Imperfecti. *Proc. Linn. Soc. Lond.* 1942–43. **1**, 34.

Muller, A. S. & Chupp, C. (1936). Uma segunda contribuiçao a's *Cercosporae* de Minas Geraes. *Arch. Inst. Biol. veg., Rio de J.* **3**, 91–98.

Yamamoto, W. (1934). Cercospora–Arten aus Taiwan (Formosa). II. *J. Soc. trop. Agric., Taiwan*, **6**, 599–608.

(*Accepted for publication* 30 *March* 1956)

Trans. Brit. mycol. Soc. **40** (2), 263–276 (1957).

NOTES ON BRITISH AGARICS 1–5 (OBSERVATIONS ON THE GENUS *COPRINUS*)

By PETER D. ORTON

(With 9 Text-figures)

(1) *Coprinus* section *Lanatuli* sensu Kühner & Romagnesi is discussed. (2) *C. jonesii* Peck, *C. funariarum* Metrod, and *C. lagopus* var. *sphaerosporus* Kühner & Josserand are shown to be synonyms of *C. lagopides* Karsten, a species distinct from *C. lagopus* (Fr.) Fr. sensu auct. non Buller. (3) *C. episcopalis* sp.nov. (section *Impexi* Romagn.) is described. (4) *C. gonophyllus* Quél. and *C. pellucidus* Karsten are recorded for Britain. (5) *C. filiformis* B. & Br. is redescribed and assigned to section *Impexi*.

(1) *Coprinus* section *Lanatuli* sensu Kühner & Romagnesi

Coprinus species are at present grouped primarily on the presence or absence of a veil and its macro- and micro-characters when present, a development of Fries's system. Fries (1874) used the macro-characters of the veil in conjunction with the mode of deliquescence of the cap, and proposed a section *Lanatuli* for species with the cap not becoming revolute, a veil forming separable white or greyish scales on the cap, and no ring. He included in this section: *C. lagopus*, *C. narcoticus*, *C. radiatus*, *C. macrocephalus* and three species not recognized to-day.

Lange (1935–40) divided the species of *Coprinus* into three groups: (*a*) with veil composed of ± cylindrical cells; (*b*) with veil composed of ± globose cells; (*c*) veil absent. Developing this system Kühner & Romagnesi (1953) proposed *Lanatuli* for a well-defined group of small to medium species with a veil composed of ± cylindrical hyphae, often slightly narrower at the cross-walls, 10–40 (60) μ in diam. and forming white or greyish separable scales on the cap, as opposed to the section *Impexi* macroscopically similar but with the veil composed of ± cylindrical narrower hyphae not constricted at the cross-walls (1·5) 3–10 μ in diam. and also other sections with the veil composed at least in part of globose cells or with the veil absent. Of the above-mentioned Friesian species *C. lagopus* and *C. radiatus* belong to *Lanatuli* sensu Kühner & Romagnesi, and also *C. macrocephalus*, figured and described by Lange (1935–1940, Pl. 158B) and Moeller (1945, fig. 72) who do not give measurements for the cells of the veil but which measured 50–100 × 8–20 μ in specimens collected recently by me. (Type specimens are not available at either Herb. Kew. or the British Museum (Nat. Hist.), but a later collection from the Berkeley Herbarium in Herb. Kew. has spores agreeing in size and shape with the measurements given by Lange and Moeller) whilst the unrelated *C. narcoticus* has a veil of globose cells. In addition to *C. lagopus* and *C. radiatus*, Kühner & Romagnesi include in the section *Lanatuli*, *C. cinereus* (as *C. macrorhizus*—

but the valid name for this species is *cinereus*, the only one given by Fries in the *Systema*), and *C. pseudoradiatus*, a species allied to *C. radiatus*. They also include *C. lagopus* var. *sphaerosporus*, differing from the type in spore size and shape.

There has been much confusion over the interpretation of the species in this section. *C. radiatus* has been interpreted in two ways, differing mainly in spore size: that of Lange with spores 11–14 × 6–7 μ (also adopted by Kühner & Romagnesi) and that of Rea (and probably also Cooke) with smaller spores 7–9 × 4–5 μ, which can be referred to *C. pseudoradiatus*. In addition to the confusion of *C. lagopus* and *C. lagopides* and its various synonyms (see (2) below), fungi growing on dung have sometimes been referred to *C. lagopus*, particularly in Britain (e.g. Buller (1924), whose *C. lagopus* is *C. radiatus* sensu Lange); this, however, seems wrong, since Fries (1874) gives 'in damp shady woods' or, for a slender form, 'in stony places' as habitats for *C. lagopus*. Such dung fungi are more correctly referred to *C. cinereus* if with cap 10 mm. or more high before expanding (rootless forms of which often resemble *C. lagopus* except for the thickened stembase), or to *C. radiatus* or *C. pseudoradiatus* if with cap less than 10 mm. high before expanding.

It is proposed, therefore, for the present to maintain the following six species in the section *Lanatuli* sensu Kühner & Romagnesi, all of which are found in Britain: *C. lagopus* (Fr.) Fr., *C. lagopides* Karsten, *C. cinereus* (Schaeff. ex Fr.) S. F. Gray (syn. *C. fimetarius* [L.] Fr.), *C. macrorhizus* (Pers. ex Fr.) Rea, *C. macrocephalus* (Berk.) Berk., *C. radiatus* (Bolt. ex Fr.) S. F. Gray sensu Lange, *C. pseudoradiatus* Kühner & Josserand (syn. *C. radiatus* (Bolt. ex Fr.) S. F. Gray sensu Rea). For a key to these species see p. 270.

(2) *Coprinus lagopides* Karsten

C. lagopides, as described by Karsten (1882), resembles *C. lagopus* (Fig. 1), but has a larger cap, small roundish ovoid spores and the habitat was given as 'in populetis'. *C. jonesii* Peck (1895) is from the description also similar macroscopically to *C. lagopus*, but has spores 'broadly elliptical 0·0003 in. to 0·00035 in. [8–9 μ] long, 0·00025 in. [6·5 μ] broad, with an apiculus at one end'. Neither *C. lagopides* nor *C. jonesii* has been mentioned in recent literature, although *C. lagopides* was compiled by Rea (1922) and Pearson & Dennis (1948), and is described in a notebook of the late A. A. Pearson in Herb. Kew.

More recently *C. funariarum* Metrod (1937) was described as a rather small species belonging to the section *Lanatuli*, but growing in moss on burnt ground and with small subglobose spores less than 10 μ long. Kühner & Josserand (1944) described *C. lagopus* var. *sphaerosporus* apparently differing only from the type in spore size and shape, the habitat being given as on burnt ground or in a muddy ditch. A comparison of these four taxa (Table 1) shows that, although there is variation in size the following features are common to all: veil on cap of white or greyish scales (given as consisting of hyphae more than 10 μ in diam. in the modern descriptions), white floccose or scaly stem and roundish spores less than 10 μ long.

Examination of a number of collections of '*C. lagopus*' in recent years has led to the conclusion that there are two macroscopically similar fungi, both with hyphae of the veil more than 10 μ in diam: (i) one growing in leaves or about stumps in woods or shady places, of small to medium size, with elliptical spores more than 10 μ long; and (ii) one growing almost always on burnt ground or wood, variable in size but often more robust than (i), with smaller subglobose or oval lenticular spores less than 10 μ long. All

Fig. 1. *Coprinus lagopus*; *a*, fruit-body, Netley Heath, Surrey, 28 November 1954, × 1; *b*, young fruit-body (section); *c*, expanded papery fruit-body, Netley Heath, Surrey, 8 October 1954, × 1; *d*, spores, Netley Heath, Surrey, 28 November 1954, × 2000.

characters given by Karsten for *C. lagopides* are applicable to (ii), except that burnt ground is not indicated as the habitat; it is, however, quite possible that Karsten (and also later observers) may have collected his fungus from old burnt soil not recognized as such, and further observations in the field are necessary to show whether this fungus is always carbonicolous or not—in the author's experience, about twenty collections, it has always been so.

Fungus (ii) has been found to vary considerably in size; in open mossy burnt places it is often quite small (such as that described by Metrod as *C. funariarum*), but in shady places it may attain a much greater size,

Table 1. *Comparison of the original descriptions of* C. lagopides *Karsten*, C. jonesii *Peck*, C. funariarum *Metrod, and* C. lagopus *var.* sphaerosporus *Kühner & Josserand*

	C. lagopides	*C. jonesii*	*C. funariarum*	*C. lagopus* var. *sphaerosporus*
Cap	40–70 mm., campanulate, becoming grey with livid disk, with separable white scales of agglomerated hyphae, radially sulcate, torn, subrevolute	1–2 in. [25–50 mm.] broad, campanulate, becoming broadly convex or expanded, very blunt or truncate at the apex when young, everywhere covered with tawny grey or pale cervine floccose scales wholly or partly disappearing with age, revealing the striate surface beneath, margin becoming split or revolute	Up to 20 mm., at first ovoid, ochraceous grey, darker at disk, shining below the thick white floccose veil, almost completely striate	15–20 mm. high, elliptic or ovoid, greyish or smoky black when expanded, densely shaggy with fine pointed reflexed whitish scales, those at disk tinged pale greyish brown, strongly sulcate over gills
Gills	Crowded, free, linear, black	Crowded, free, linear, at first white or whitish becoming black	Very crowded, free, fairly wide, white then violaceous then black	Very crowded, free or even remote, narrow, dark grey brown then quickly blackening
Stem	Up to 170 × 3 mm., slightly attenuated above, white, floccose, shining when dry, apex flocculose pruinose, hollow	2–3 in. [50–75 mm.] × 2–3 lines [4–6 mm.], equal or slightly tapering upwards, white, minutely floccose, hollow	20–30 × 4 mm. in the middle, attenuated upwards, slight central thickening persisting, covered by the white veil below as fine white scales	Up to 100 mm. × 3·5–5 mm. at base, 2·5 mm. at apex, gradually attenuated upwards, not bulbous, silvery white, distinctly shaggy (short recurved fibrils), hollow, very fragile
Spores	Oval or oval globose, 6–8 × 5–6 μ, black, impellucid s.m.	Broadly elliptical, 0·0003–0·00035 in. × 0·00025 in. [8–9 × 6·5 μ], with an apiculus at one end	Citriform with wide germ-pore, 7–8·5 × 5·5–6 μ, apiculus large	Subglobose with distinct pore, angular at base in outline, 7·5–9 × 5·5–7 μ, with distinct projecting apiculus
Cystidia	No information	No information	Marginal—no information; facial—vesiculose, 75–100 × 50–60 μ	Marginal—variable, 40–100 μ long, very numerous; facial—cylindric, elliptic or obtusely lanceolate, attached by one end only, 80–160 × 30–45 μ
Hyphae of cap scales	No information	No information	Mostly elliptic, 50–90 × 20–32 μ	± Elongate, some shorter, terminal cells lanceolate or attenuated but obtuse, 20–45 μ wide
Habitat	Gregarious under poplars	In a cellar	In troops among *Funaria hygrometrica* on burnt soil	In muddy ditch and on burnt soil

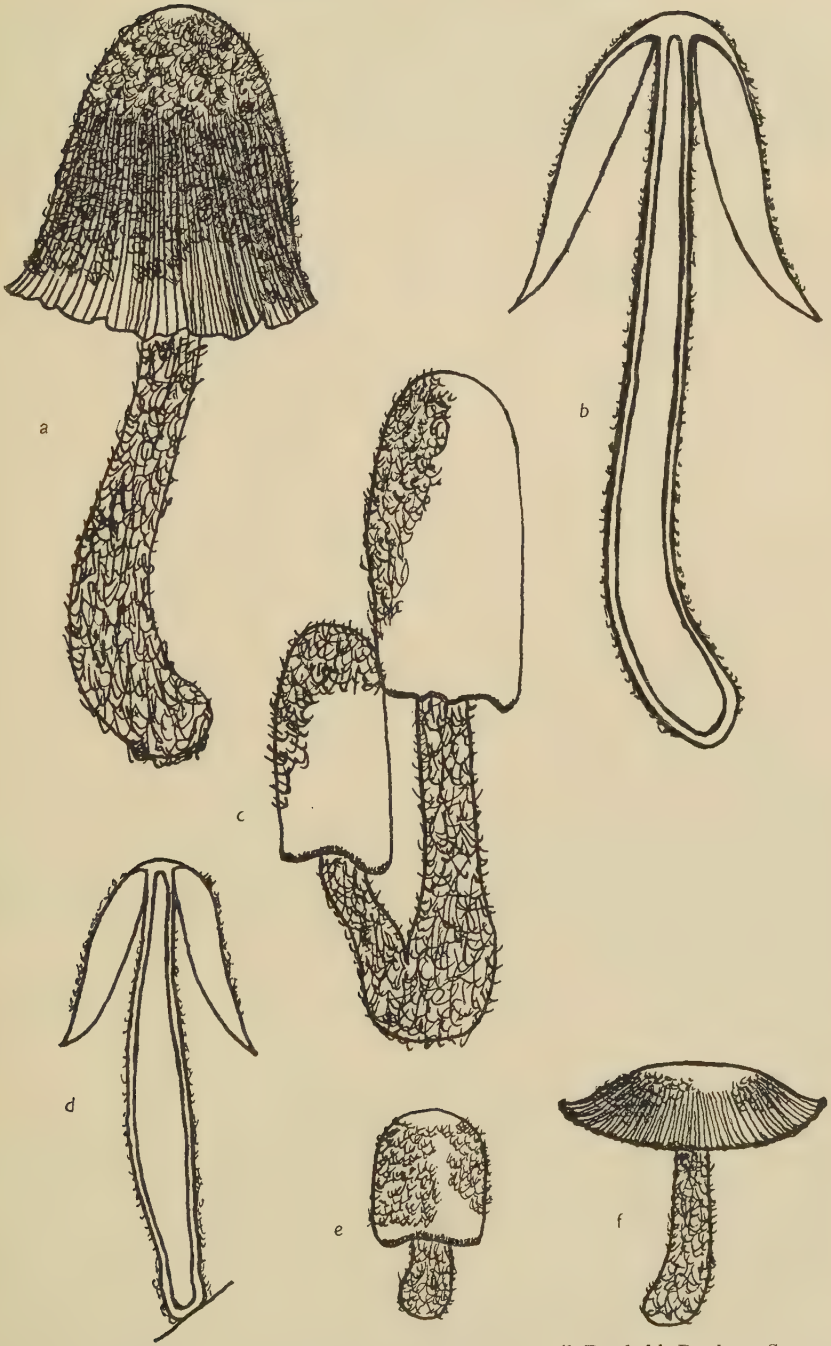


Fig. 2. *Coprinus lagopides*; *a*, fruit-body; *b*, in section, on burnt soil, Dunkeld, Perths, 5 September 1954; *c*, fruit-body, on rich burnt soil, Polesden Lacey Woods, Surrey, 3 October 1954; *d*, fruit-body, at base of burnt stump, Juniper Hall, Surrey, 16 November 1952; *e*, young, and *f*, expanded fruit-body, mossy burnt patch in field, Bramshaw, Hants, 11 September 1954. (All × 1.)

e.g. cap up to 57 mm. high and 37 mm. broad before expanding. Both extremes have spores of almost identical size and shape (Fig. 3) and other micro-characters similar and it seems desirable to keep fungus (ii) with smaller spores as a distinct species, especially as it is mainly (possibly always) carbonicolous. Since neither Fries nor Karsten gave measurements for the

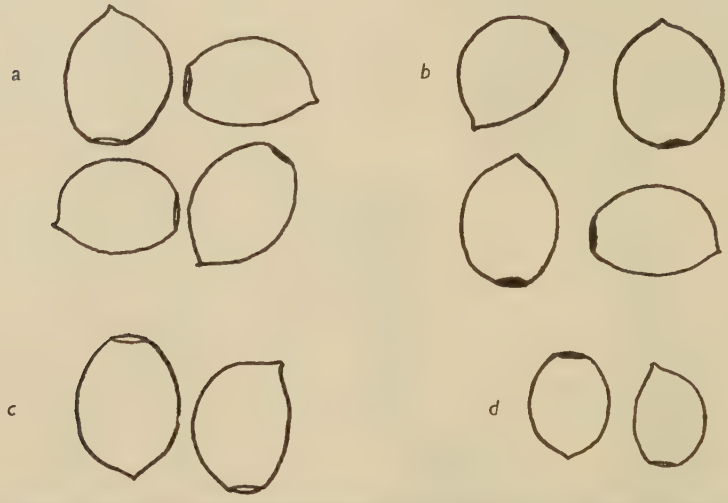


Fig. 3. *Coprinus lagopides*; *a* spores from Bramshaw, Hants (Fig. 2*f*); *b*, spores from Dunkeld, Perths (Fig. 2*a*). *Coprinus lagopus* var. *sphaerosporus*; *c*, spores (after Kühner & Josserand). *Coprinus funariarum*; *d*, spores (after Metrod). (All × 2000.)

hyphae of the veil for *C. lagopus* and *C. lagopides*, and *C. lagopus* sensu Fries is assumed to have had hyphae more than 10 μ wide, it seems equally reasonable to assume that *C. lagopides* sensu Karsten also had hyphae more than 10 μ wide, and other characters being in agreement it is proposed to maintain *C. lagopides* Karsten as the valid name for (ii) and *C. lagopus* (Fr.)Fr for (i). *C. lagopides* is therefore redefined as follows:

Coprinus lagopides Karsten, *Helsingfors Meddelanden*, 9 (1882), 48; *Icon. Select. Hymen. Fenniae*, pl. IX, f. 50

C. jonesii Peck, *Bull. Torr. bot. Cl.* **22** (1895), 206. (Type not examined.)
C. funariarum Metrod, *Bull. Soc. mycol. Fr.* **53** (1937), 346.
C. lagopus var. *sphaerosporus* Kühner & Josserand, *Bull. Soc. mycol. Fr.* **60** (1944), 31.

Cap 17–57 mm. high, 10–35 mm. in diam., at first cylindrical, campanulate or rarely conical then expanding and becoming split or torn or ± revolute at the margin (Fig. 2), white or whitish then grey with apex tinged buff or pale date-brown, at first entirely covered with erect or recurved white feathery scales which become greyish and are easily separable and finally disappear; margin becoming grey and striate or sulcate as the cap expands. *Gills* white then brownish- or violaceous-black, finally black, crowded, free, lanceolate, edge white at first. *Stem* 15–90 × 3·5–12 mm., equal with attenuated apex or sometimes ventricose at first, white, entirely white fibrillosely scaly, hollow, base white tomentose rarely

slightly thickened. *Flesh*, thin, concolorous, thickest at disk. *Smell* none. *Spores* subglobose or oval, lenticular, 6–8 × 5–7 × 4·5–6 μ, with conspicuous truncate germ-pore (Fig. 3). *Marginal cystidia* clavate or vesiculose 40–100 × 20–30 μ; *facial cystidia* vesiculose or slightly flask-shaped 80–160 × 25–50 μ (Fig. 4*e–f*). *Hyphae* of scales on cap ± cylindrical or elongate elliptic (40) 50–200 × (12) 20–40 μ (Fig. 4*d*); those of scales on stem similar but cells often narrower (8–20 μ in diam.).

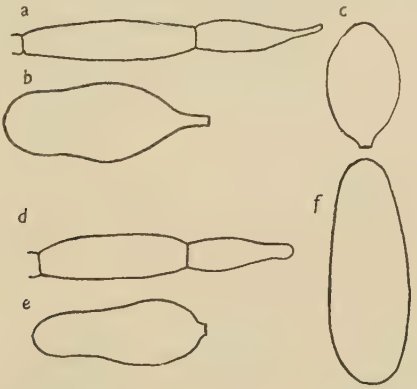


Fig. 4. *Coprinus lagopus*; *a*, hyphal cells from scale on cap; *b*, *c*, facial cystidia. *Coprinus lagopides*; *d*, hyphal cells from scale on cap; *e*, *f*, facial cystidia. (× 250.)

On burnt ground, charred wood or plaster ceilings, more rarely on (?) unburnt soil, in woods or in the open (and then often smaller), gregarious or in small clumps, less often solitary, rather common. Juniper Hall, Surrey 16 November 1952 (at base of burnt stump); Dunkeld, Perthshire, 5 September 1954 (on burnt ground); Bramshaw, Hants, 11 September 1954 (small form on open mossy burnt patch); Norbury Park, Surrey, 21 November 1954 (on old burnt stump—exsiccata in Herb. Kew.), and several other Surrey localities 1954 and 1955. Also recorded by the late A. A. Pearson on plaster ceiling, Hindhead, Surrey, 18 August 1951 and previously from other localities, and by Dr R. W. G. Dennis on burnt soil, Clophill, Beds., 12 October 1952 (as *C. funariarum*) and from plaster ceiling Bicester, Oxon., 18 March 1953.

Obs. C. lagopides is most surely recognized by microscopic examination (± subglobose spores less than 10 μ long and veil hyphae more than 10 μ in diam.), but can be distinguished in the field from *C. cinereus* by habitat and lack of swollen or rooting stem-base and from *C. lagopus* by habitat if on burnt ground or charred wood and also by certain other small points. It is often more luxurious, not only in size but also in the number of fruit-bodies in a group. It is often much whiter and more substantial in appearance, especially the smaller specimens and remains whiter for a longer time before becoming grey from the margin inwards. *C. lagopus*, on the other hand, is less variable in size, soon turns grey and appears often to be less deliquescent so that older specimens may be found with the cap completely expanded, papery and entire.

Key to Coprinus *section* Lanatuli *sensu Kühner & Romagnesi*

1. Cap very small, rarely more than 6 mm. high before expanding (may be larger in culture); stem not or barely thickened at base, not persistently scaly; on dung. **2**
1. Cap small to fairly large, more than 10 mm. high before expanding: stem thickened at base or not, often persistently white fibrillosely scaly; on dung, soil, stumps, burnt ground, charred wood, or plaster ceilings. **3**
2. Spores 11–14 × 6–7 μ, elliptic or slightly phaseoliform; scales on cap white or whitish at first. **C. radiatus** (Bolt. ex Fr.) S. F. Gray sensu Lange.
2. Spores 7–9 × 4–5 (5·5) μ, elliptic not phaseoliform; scales on cap grey from the first. **C. pseudoradiatus** Kühner & Josserand.
3. Spores 11–14(17) × 7–9 μ, broadly elliptic; stem fibrillose-pilose or minutely flocculose at first, soon smooth; (on dung, rotten straw or sawdust). **C. macrocephalus** (Berk.) Berk.
3. Spores mostly less than 7 μ broad; stem ± persistently flocculose or white fibrillose-scaly. **4**
4. On dung (also found on hop-waste); stem bulbous at base (often markedly so), often rooting; spores elliptic 10–11·5 × 6–7 μ. **C. cinereus** (Schaeff. ex Fr.) S. F. Gray.
4. Not on dung; stem not or barely swollen at base, not or rarely rooting; spores elliptic or subglobose. **5**
5. Spores (11) 12–14 × 6–6·5 μ, elliptic (Fig. 1*d*); cap with scales soon greyish, sometimes found papery and ± entire when fully expanded; among leaves, in soil or about stumps in woods or shady places, often solitary or in twos or threes, more rarely subcaespitose. **C. lagopus** (Fr.) Fr.
5. Spores 6–8 × 5–7 × 4·5–6 μ, subglobose or oval, lenticular (Fig. 3): cap with scales often remaining white or whitish for some time, usually deliquescing and becoming torn or revolute, rarely found papery when expanded; on burnt ground, charred wood or plaster ceilings, more rarely on (?) unburnt soil, often gregarious or in small clumps, more rarely solitary; occurs both in woods and out in the open (when it may be smaller). **C. lagopides** Karsten

(3) **Coprinus episcopalis** sp.nov. (Figs. 5, 6)

Pileus 20–30 mm. altus, 10–18 mm. latus dein ad 55 mm. expansus, e cylindrico vel ± conico campanulatus explanatusque, primo ubique albofloccosus dein squamis superficialibus latis albis rufescentes vel fuscescentes variegatus, margine cinereo striato sulcatoque disco albido vel alutaceo denique laeve. Lamellae ex albo cinerascentis nigrisque, liberae, confertae, linearis haud ventricosis, acie primo alba. Stipes primo 30–40 mm. longus, 5–9 mm. crassus (ad basim 8–15 mm.), dein 60–95 mm. longus, albus, ± sericeo-striatus, fistulosus, semper bulbosus saepe marginato-bulbosus. Caro pilei tenuis concolore, stipitis alba. Odor nullus dein paulum stercorarius. Sporae mitriformae, 7·5–10 × 6·5–8 × 5·5–6·5 μ poro truncato. Cheilocystidiae clavatae vel vesiculosae vel paulum irregulares, 30–40 (60) × 16–30 μ. Pleurocystidiae magnae, vesiculosae ad apicem saepe angustiores, 90–180 × 20–60 μ. Squamis pilei ex hyphis ramosis angustis ad apicem pilei tunicis crassis fuscescentibus (3) 4–8 (9) diam. composita. In fagetis solo calcareo solitarius. *C. picaceo* proximus sed minor et sporis insignis. Mickleham Downs, Surrey, 20 November 1953 (typus, Herb. Kew.)

Cap 20–30 mm. high, 10–18 mm. in diam. when young, up to 55 mm. in diam. when expanded, cylindrical or ± conical at first, then campanulate, umbonate or not, finally expanded with margin split and revolute (Fig. 5), at first entirely covered by the white veil, then grey and striate or sulcate to smooth pale or pallid disk, veil breaking up into adpressed separable whitish patches, those on disk often tinged pinkish or yellowish brown. *Gills* white then mottled grey and black, finally black, free, crowded, linear or ± lanceolate, edge very white floccose at first. *Stem* 95 mm.,

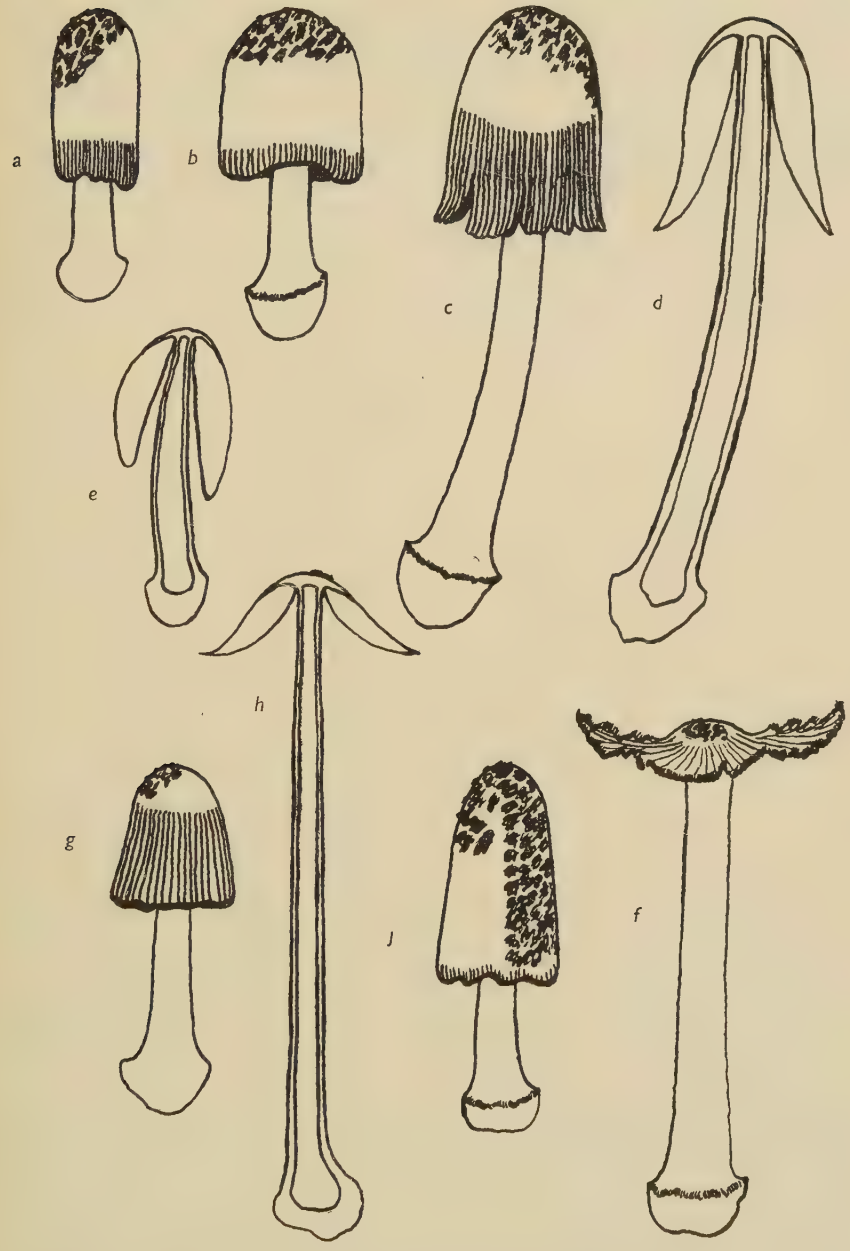


Fig. 5. *Coprinus episcopalis*; *a–c*, fruit-bodies (*d*, *e*, in section); *f*, old fruit-body, Mickleham Downs, Surrey, 20 November 1953; (Type); *g*, *h* (section), Norbury Park, Surrey, 14 October 1953; *j*, type locality, 1 October 1954. (× 1.)

white, base always bulbous often marginately so, ± white silky striate, hollow and fragile when elongated. *Flesh* thin, concolorous in cap, 30–40 × 5–9 mm. (8–15 mm. in bulb) elongating as cap expands to 60– white in stem. *Smell* none, then rather dung-like. *Spores* mitriform, lenticular, 7·5–10 × 6·5–8 × 5·5–6·5 μ, with truncate germ-pore (Fig. 6*a*). *Marginal cystidia* clavate to vesiculose or slightly irregular, 30–40 (60) × 16–30 μ (Fig. 6*b*). *Facial cystidia* large, vesiculose, often narrowed at apex,

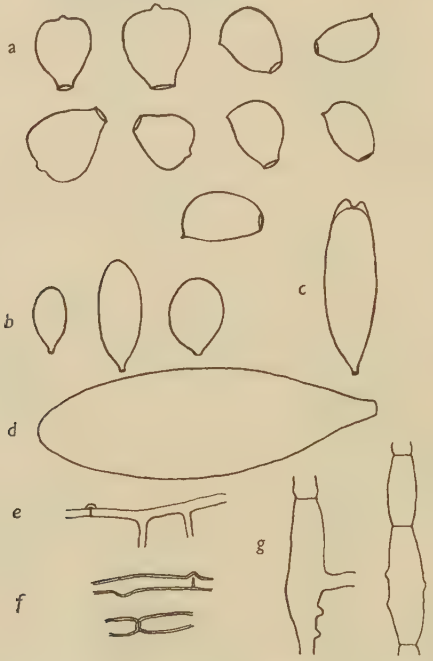


Fig. 6. *Coprinus episcopalis*; *a*, spores; *b*, marginal cystidia; *c*, *d*, facial cystidia; *e*, thin-walled hypha of veil; *f*, thick-walled hyphae of veil; *g*, hyphae from cap below veil; all from Type. (*a*, × 1000; *b–g*, × 250.)

90–180 × 20–60 μ (Fig. 6*c*,*d*). *Veil* on cap of hyaline branched clamped narrow hyphae (3) 4–8 (9) μ in diam. (Fig. 6*e*), those on disk often with thick brownish walls (Fig. 6*f*). Hyphae of cap cuticle ± cylindric up to 20 μ in diam. (Fig. 6*h*).

Among leaves of *Fagus* on chalky soil, usually solitary. Norbury Park, Surrey, 14 October 1953; Mickleham Downs, Surrey, 20 November 1953 (type collection), and subsequently.

Obs. Resembles *C. picaceus* (Bull. ex Fr.) S. F. Gray in having a bulbous stem, large flat separable scales on the cap and a disagreeable smell when mature, but is much smaller and has characteristic spores resembling those of *C. angulatus* Peck (= *C. boudieri* Quél.) and is probably the species referred to by Kühner & Romagnesi (1953, p. 391, note 11) as a species 'à rechercher'. Older specimens are often covered with flies presumably attracted by the smell. *C. picaceus* is about twice the size of *C. episcopalis* and has larger elliptical spores 14–17 × 10–12 μ.

(4) *Coprinus gonophyllus* Quél. and *C. pellucidus* Karst., two new British records

C. gonophyllus Quél., *Ann. sci. nat. Bordeaux*, 1884, pl. 1, fig. 2; Romagnesi, *Rev. Mycol.* **10** (1946), 79.

Cap 5–10 mm. in diam., up to 15 mm. when expanded, at first like a small white round or oval ball, entirely covered by the white veil, then expanding with separable flat scales which often become brownish in places (resembling *C. picaceus* in miniature), margin becoming grey and striate or sulcate. *Gills* whitish then purplish-brown to purplish-brown-black, free, not crowded, remarkably broad when the cap expands and almost triangular in shape, edge at first white floccose.

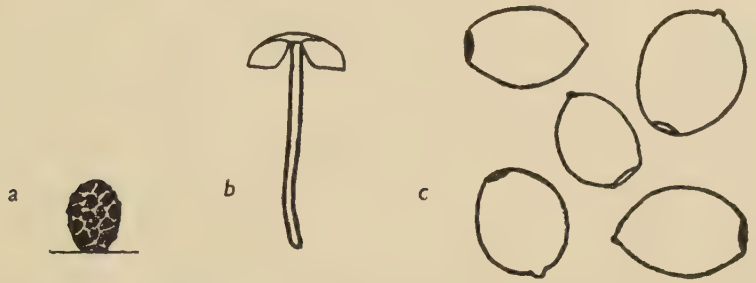


Fig. 7. *Coprinus gonophyllus*; *a*, young fruit-body (after Pat.), × 1; *b*, section of fruit-body (after Pat.), × 1; *c*, spores, Oxshott, Surrey, 16 October 1954, × 2000.

Stem 15–30 × 1·5–2 mm., attenuated at apex, ± thickened or bulbous at the base, white, ± smooth. *Flesh* thin, concolorous. *Smell* none. *Spores* subglobose lenticular, 6–7·5 (8) × 5·5–6·5 × 4·5–5 μ, apiculus not prominent but germ-pore conspicuous (Fig. 7*c*), dark violaceous-brown in mass. *Marginal cystidia* ± clavate, up to 45–50 × 11–16·5 μ. *Facial cystidia*, none seen. *Hyphae* of veil on cap narrow 1·5–3 μ in diam., branched, some with thicker walls or slight protuberances.

On burnt ground Rothamsted, Herts (leg. Miss M. Holden) 22 May 1953: on burnt ground Oxshott Heath, Surrey, 16 October 1954: also reported by Dr R. W. G. Dennis from plaster ceilings in London area, 1953 (Herb. Kew.).

Obs. C. gonophyllus belongs to the same section (*Impexi* Romagn.) as *C. picaceus* with the scales on the cap from the veil composed of narrow hyphae, but is easily distinguished from this species and others in this section by its small size, broad gills, spore characters and habitat (Fig. 7).

C. pellucidus Karsten, *Helsingfors Meddelanden*, **9** (1882), 61.

Cap 0·5–4 mm. high, campanulate to hemispherical, then expanding and splitting (up to 9 mm. in diam.), whitish with date-brown apex to completely date-brown at first, becoming grey with slight reddish brown disk, minutely pubescent s.l., margin ± sulcate (Fig. 8*a*). *Gills* whitish soon brownish-black, rather distant, slightly ventricose, edge white flocculose at first. *Stem* 8–50 × 0·2–0·75 mm., very slender, hyaline, minutely pruinose

s.l., base slightly coloured (pallid) and with a few whitish mycelial strands. *Smell* none. *Spores* elliptic rather narrow, 8–10 × 4–4·5 μ, with germ-pore (Fig. 8*f*). *Marginal cystidia* vesiculose to lageniform, 20–30 × 10–20 μ (Fig. 8*d–e*); no facial cystidia seen. 'Hairs' on cap ± lageniform with ± pointed apex, 40–80 × 10–14 (16) μ (Fig. 8*b*); similar cells on stem (Fig. 8*c*).

On old cow dung, near Juniper Hall, Mickleham, Surrey, 29 October 1954 (Herb. Kew.); cow-dung Tomich, Strathglass, Inverness, 19 August 1955; cow dung Rothiemurchus, Aviemore, Inverness, 25 August 1955.

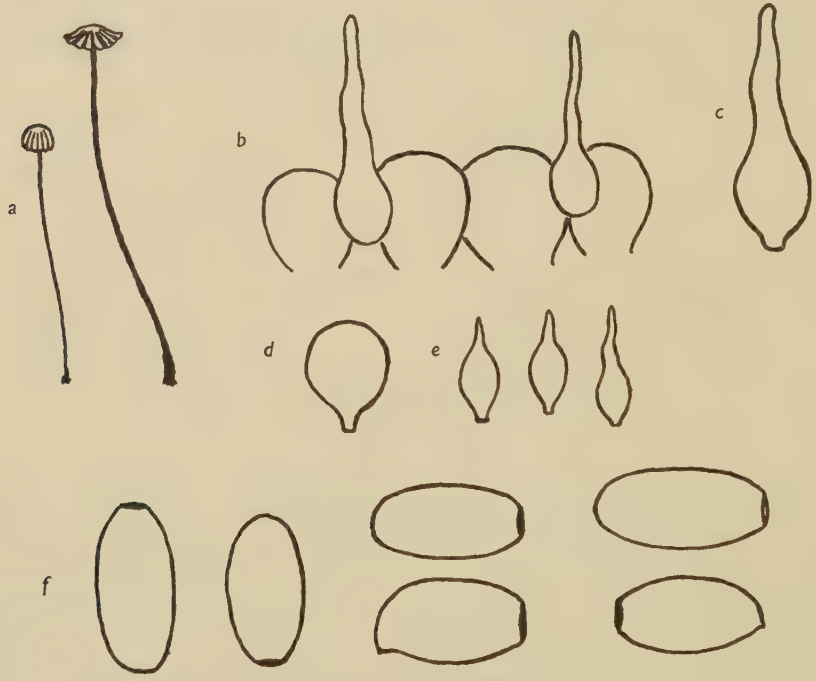


Fig. 8. *Coprinus pellucidus*. *a*, fruit-bodies, × 1; *b*, cells of cap with dermatocystidia, × 500; *c*, cystidium from stem, × 500; *d*, *e*, marginal cystidia, × 500; *f*, spores, × 2000.

Obs. C. pellucidus is one of a number of species in the section *Setulosi* Lange recently clarified by Morten Lange (1952), readily distinguished from other small members of this section by its relatively narrow spores. The sample of rather old cow dung from Mickleham was kept damp under a bell-jar and produced fruit-bodies, showing the considerable variation in size typical of species of *Coprinus* on dung, continuously over a period of 6 weeks.

(5) *Coprinus filiformis* B. & Br.

During the British Mycological Society foray at Bristol, September 1955 a number of small Coprini, which could not be referred to any species described in available literature, were collected from bare soil along the side of a path through the wood at the Coombe, Cleeve, Somerset. Further investigation showed, however, that the collection agreed macro-

scopically with *Coprinus filiformis* B. & Br. as originally described, and also with the species as depicted by Cooke (1881–90, pl. 674/686B). The habitat given by Berkeley & Broome, 'on the ground in a wood' and the date of occurrence, 'Sept. 19', are also in agreement. No type material of *C. filiformis* appears, however, to be available, either at Herb. Kew. or the British Museum (Nat. Hist.) and it is therefore proposed to redefine the species as follows:

Coprinus filiformis B. & Br., *Ann. Mag. nat. Hist.* **7**, Ser. 3, (1861), 379, pl. 15, fig. 8. (Fig. 9).

Cap 2–6 mm. in diam. before expanding, elongate ± cylindrical at first, then convex often slightly umbonate becoming expanded with upturned edge (Fig. 9*a–b*), pale whitish grey, becoming deeper grey with darker

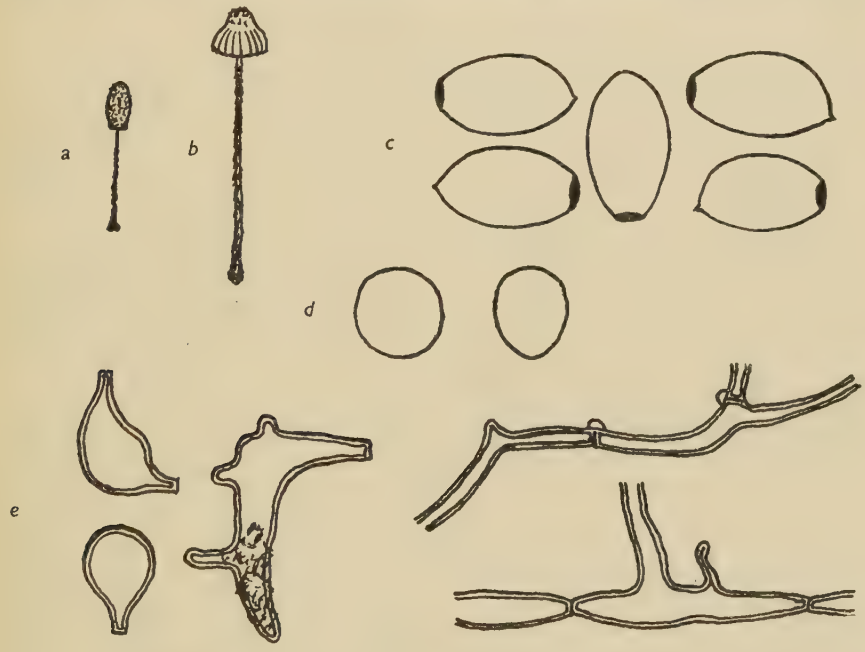


Fig. 9. *Coprinus filiformis*. *a*, *b*, fruit-bodies, × 1; *c*, spores, × 2000; *d*, thin-walled cells of veil on cap, × 500; *e*, thick-walled cells of veil on cap, × 500.

horn- or bistre-tinged disk, margin becoming wrinkled-sulcate. *Gills* whitish then greyish finally black, soon free, rather distant. *Stem* 15–40 × 0·25–1 mm., very slender and fragile, minutely hairy-pubescent especially at the base, which is often thickened or almost disk-like. No smell noticed. *Spores* elliptic with germ-pore, 8–10 × 4·75–5·5 μ, date-brown s.m. (Fig. 9*c*). *Cystidia* not investigated (none seen). *Hyphae of veil* of two kinds: (*a*) ± elliptic or globose, thin-walled, 20–40 μ (Fig. 9*d*); (*b*) ± cylindric, often diverticulate, thick-walled, 4–6 μ in diam., sometimes broader and irregularly shaped (Fig. 9*e*) and with walls brownish and encrusted s.m. (in water), with clamps.

Scattered at intervals on bare soil at side of shady path through Cleeve Coombe, Somerset, 12 September 1955 (Herb. Kew.).

Obs. A rather distinct but insignificant and easily overlooked species belonging to the section *Impexi* Romagn. distinguished from other small members of this section by habitat, possession of two kinds of cells in the veil and spore characters.

I am greatly indebted to the Nuffield Foundation for a grant to work on agarics and also to Dr R. W. G. Dennis of the Kew Herbarium for much helpful advice and free access to literature and material.

REFERENCES

Berkeley, M. J. & Broome, C. E. (1861). Notices of British Fungi. *Ann. Mag. nat. Hist.* **7**, Ser. 3, 373–382.

Buller, A. H. R. (1924). *Researches on fungi*, **3**, 299–327. London.

Cooke, M. C. (1881–1890). *Illustrations of British Fungi.* London.

Fries, E. (1874). *Hymenomycetes Europaei*, p. 329. Uppsala.

Karsten, P. A. (1882). *Symbolae ad Mycologiam fennicam*, IX. *Helsingfors Meddelanden*, **9**, 39–67.

Kühner, R. & Romagnesi, H. (1953). *Flore Analytique des Champignons Supérieures*, pp. 374–391. Paris.

Kühner, R. & Josserand, M. (1944). Études de Quatre Coprins du groupe '*lagopus*'. *Bull. Soc. mycol. Fr.* **60**, 19–37.

Lange, J. E. (1935–40). *Flora Agaricina Danica*, **4**, 104–119. Copenhagen.

Lange, M. (1952). Species concept in the genus *Coprinus*. *Dansk. Bot. Arkiv.* **14**, 1–164.

Metrod, G. (1937). *Coprinus Funariarum* nov.sp. *Bull. Soc. mycol. Fr.* **53**, 346–349.

Moeller, F. H. (1945). *Fungi of the Faeroes, Part I, Basidiomycetes*, pp. 166–167.

Pearson, A. A. & Dennis, R. W. G. (1948). Revised list of British Agarics and Boleti. *Trans. Brit. mycol. Soc.* **31**, 145–190.

Peck, C. H. (1895). New species of fungi. *Bull. Torrey. bot. Cl.* **22**, 198–211.

Quélet, L. (1884). *Ann. Sci. nat. Bordeaux.*

Rea, C. (1922). *British Basidiomycetae*, pp. 511–512. London.

Romagnesi, H. (1945). Quelques Coprins. *Rev. Mycol., Paris*, N.S., **10**, 73–89.

(*Accepted for publication* 13 *June* 1956)

Trans. Brit. mycol. Soc. **40** (2), 277–278 (1957).

THIELAVIA INDICA SP.NOV.

By S. B. CHATTOPADHYAY and C. DAS GUPTA

State Agricultural Research Institute, Calcutta, India

(With 1 Text-figure)

Thielavia indica sp.nov. isolated from the soil of paddy fields of State Agricultural Farm, Chinsurah, India, is described. It differs from other species of *Thielavia* in having sixteen ascospores in each ascus instead of eight.

Thielavia indica sp.nov. (Fig. 1)

Coloniae moderate crescentes in 'potato-dextrose agar', primum albidae, tum evadentes pallide, roseae vel pallide roseo-violaceae. Hyphae diametri variabilis, 0·8–4 μ, septate, ramosae, tenuiores quidem hyalinae, latiores vero olivaceae colore. Cleistothecia immersa in medium, globosa vel subglobosa, sessilia, absque ullo appendice, nigra, parietibus pseudo-parenchymaticis, membranacea, 1–3 seriebus constituta, magnit. 178·8–650 μ, longitudine, 162·5–405·8 μ latitudine. Asci plures, dispersi ovales vel pyriformes, non-pedicellati, tenuibus parietibus praediti, magnit. 14·8–12·8 × 3·2–5·6 μ. Ascosporae 16 in singulis ascis, irregulariter dispositae, ovatae vel oblongae, semel cellulatae, fusce brunneae, crassis parietibus praeditae, leves, magnit. 4·8–12·8 × 3·2–5·6 μ.

Typus lectus in humo in orizetis in State Agricultural Farm, in loco Chinsurah, in provincia West Bengal in India (Herb. I.M.I. 57849).

While investigating the soil fungi of paddy fields at the State Agricultural Farm, Chinsurah (West Bengal, India), a species of *Thielavia* was isolated. As a study of the literature failed to reveal any description that matched this fungus, which differed from the other species of *Thielavia* in having sixteen spores in the ascus, it is described as new.

Colonies on potato-dextrose agar at 25° C. are slow growing and moderately spreading. The aerial hyphae are floccose at first, but later become matted. At first white, later light pinkish to light pinkish violet. Light pinkish violet concentric zones are evident on potato-dextrose agar within 96 hr. at 25° C. and cleistothecia appear in these zones within 120 hr. The cleistothecia are embedded in the medium and preponderate in the inner zones. *Hyphae* 0·8–4·0 μ, septate, and branched. The thinner hyphae are usually hyaline, while the broader ones are very light olivaceous in colour. *Cleistothecia* are embedded, globose to subglobose, sessile, and without appendages. The wall is black, membranous, and pseudo-parenchymatous with one to three layers of cells. Cleistothecia measure 178·8–650 μ in length and 162·5–405·8 μ in breadth. Each contains numerous irregularly arranged asci. *Asci* are oval to pyriform, non-pedicillate, thin-walled, light-brown, and 14·8–20·4 × 11·4–18·4 μ (averaging 17·8 × 14·5 μ). They deliquesce at maturity. *Ascospores*, sixteen per ascus, are irregularly arranged, oval to oblong, one-celled, thick-walled, smooth, dark-brown,

4·8–12·8 × 3·2–5·6 μ (averaging 8·8 × 4·4 μ). One or two ascospores in an ascus may occasionally become enlarged and attain a length of 14·8 μ.

The type culture has been deposited at the Commonwealth Mycological Institute, Kew, England and a subculture has been placed in the Indian Type Culture Collection of Fungi, Indian Agricultural Research Institute, New Delhi.

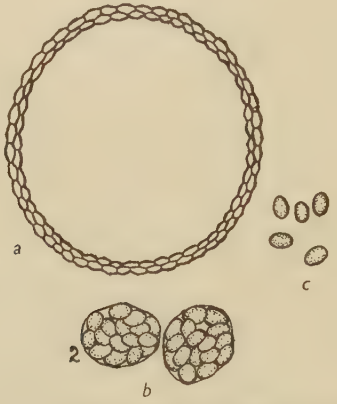


Fig. 1. *Thielavia indica* sp.nov. *a*, Pseudoparenchymatous wall of the cleistothecium (× 400); *b*, asci with 16 ascospores (× 550); *c*, ascospores (× 550).

The only other Indian record of a species of *Thielavia* is that by Chattopadhyay (1954) who reported *Thielavia terricola* (Emmons) Gilman & Abbott.

We express our thanks to Mr E. W. Mason of the Commonwealth Mycological Institute, Kew, for his help in the specific identification. Our thanks are also due to Rev. Fr. H. Santpau, St Xavier's College, Bombay, for rendering the description into latin.

REFERENCE

Chattopadhyay, S. B. (1954). Two new additions to the knowledge of Indian Ascomycetes. *Indian Phytopath.* **7**, 69–71.

(*Accepted for publication* 5 *July* 1956)

Trans. Brit. mycol. Soc. **40** (2), 279–282 (1957).

OBSERVATIONS ON GASTEROMYCETES, 5.

STUDIES ON TYPE MATERIAL NO. 3, *GEASTRUM RECOLLIGENS* (WOODW. EX SOW.) S. F. GRAY

By J. T. PALMER

5 *Station Road, Woodley, Stockport, Cheshire*

(With 1 Text-figure)

Geastrum recolligens (Woodw. ex Sow.) S. F. Gray is shown to be the valid name for *G. mammosum* Chev., and Sowerby's specimen is re-described and designated as the type. The literature and the British records are discussed.

Historical

Bryant (1782) recognized only two stellated puff-balls, *Lycoperdon fornicatum* and *L. stellatum*. However, in his discussion of the habit of *L. stellatum*, he referred to a form represented by his fig. X, said to be found on the slopes of banks, and wrote '. . . the fleshy coat of these small plants is so very thin, that it never flakes into the form of a cup, like fig. III. If a fresh and perfect plant of this size be laid upon a window in the sun, in a short time the rays will be in motion, and endeavouring to lay themselves flat; which position once gained, and the plant is suffered to remain in the sun an hour or two longer, they will incline upward, and at last inclose the head in the same manner they did before they first expanded, and the under cuticle will drop off.' Fig. X shows a small geaster with recurved rays, a sessile endoperidium and a determinate fimbriate mouth. Figs. III, IV, V and, possibly, VI are typical freshly expanded *Geastrum triplex* Jungh.

Woodward (1794) added *L. recolligens* to *L. coliforme* Dicks., *L. fornicatum* Huds. and *L. stellatum* L., citing as synonyms *L. volvans recolligens* Schmidel (although considering the figures to be possibly based on two species), *L. stellatum* Bulliard (*Plantes de la France*, Tab. 238) and *L. stellatum* Bryant (1782; figs. III–VI and X). He thought Bryant's fig. X an excellent representation of the small specimens, and his other figures to be very good ones of the larger geasters; hence Woodward's *L. recolligens* must also have embraced what we now call *G. triplex*. However, he described in detail the hygroscopic nature of the exoperidium, adding that the changes were quicker and more certain in the smaller specimens, as well as the silvery white colour of the under layer, the sessile endoperidium and the determinate, fimbriate mouth.

Persoon (1801) referred *L. recolligens* to *G. hygrometricum β anglicum*.

Sowerby (1809; tab. 401) validated Woodward's binomial and gave a fine figure. He described the strongly hygroscopic nature of the exoperidium and, whilst he gave no locality for his specimen, as it came from the Rev. J. Dalton, it was very likely from Yorkshire.

S. F. Gray (1821) recombined the epithet *recolligens* as *Geastrum.*

Chevalier (1826) described *G. mammosum* with a hygroscopic exoperidium, a sessile endoperidium and an acuminate mouth, citing *G. hygrometricum β anglicum* Pers. and *L. recolligens* Woodw. as synonyms, as well as the Bryant and Bulliard figures.

Fries (1829) wrote under *G. mammosum* 'Radii saepius inflexi, sed *siccitate reflexi*, valde hygrometrici', from which some authors infer that he had another species in mind. As his nomenclature has most frequently been followed, he is usually cited as the author.

Berkeley & Broome (1848) stated under *G. mammosum*: '. . . An authentic specimen of Sow. t. 401 proves to be this species.'

Smith (1873) reproduced Sowerby's figure and reported the spores from Sowerby's specimen, then in Berkeley's possession, as 'somewhat echinulate, and measure 0·00016″ diameter'. Saccardo (1888) referred this figure to *G. umbilicatum* Fr.

Hollós (1904) referred *G. mammosum* Fr. to *Lycoperdon corollinum* Batsch, which he validated and recombined as *Geaster.*

The type specimen

In Herb. Kew a single specimen glued to a sheet of paper labelled 'Herb. Berkeleyanum. M. J. Berkeley. 1879', upon which Berkeley has written (in ink) '*Geaster mammosus*, Chev.' and (in pencil) '*Lycoperdon recolligens* Sow. t. 401. Sow. Herb.'

Fig. 1. *a*, *Geastrum recolligens* (Woodw. ex Sow.) S. F. Gray, type specimen (K.), × 1; *b*, spores and capillitium of type specimen, × 1000.

The flattened *exoperidium* measures 1 × 26· × 2·2 cm. and is divided into nine narrowly acute rays, tending to subdivide, hard, woody and incurved. *Mycelial layer* absent or obscured by being glued to the sheet. *Fibrillose layer* fawnish white, smooth, thick, hard. *Fleshy layer* thin, cohering, blackish brown. *Endoperidium* appearing sessile, 1·4 × 1·2 cm. diam., somewhat torn and flattened, ruddy-fawn, smooth. *Mouth* determinate (seated in a well-defined area), conical, fimbriate, but somewhat damaged. *Capillitium* coloured, aseptate, simple, with adhering debris, central lumen

faintly apparent, up to 10·5 μ thick, tapering at both ends to 1·5 μ. *Spores* dark brown in the mass, coarsely to finely or sparsely verrucose, globose, thick-walled, 3·0–4·0–6·0 × 3·0–4·0–5·5 μ (with verrucae 3·5–5·0–6·5 × 3·0–4·5–6·0 μ).

Although it was not possible to soak out the specimen, the typical hard, woody rays and firmly adhering fleshy layer leave no doubt that the specimen belongs to the hygroscopic series of *Geastrum*.

Delimitation of the species

A study of the world literature indicates a spore range of 3–7 μ. The following measurements are noteworthy: Morgan (1889), 5–6 μ; Lloyd (1902), 3–7 μ, he stated that English specimens tend to be more uniform, i.e. 3–4 μ; Hollós (as *G. corollinum*), 3–4 μ (capillitium 4–6 μ); Petri (1909), 3–5 μ (capillitium 5–7·5 μ); Rea (1922), 3–6 μ (capillitium 4–6 μ); Coker & Couch (1928) 3–3·7 μ and for *G. argenteum* Cke., which they considered a synonym, 3·7–4·5 μ; Long & Stouffer (1948), 3–5 μ.

Staněk (1953) rehabilitated *G. hungaricum* Hollós (previously considered a synonym of *G. floriforme* Vitt.) which apparently differs by its extremely small size, *c.* 10 mm. diam. of the expanded rays, well-defined margin to the mouth, spores 4–6 μ and capillitium 2–4 μ. He distinguished it from *G. mammosum* Chev. by the latter having distinctly smaller spores averaging little more than half the size of those of *G. hungaricum*.

However, Sowerby's specimen has a spore range of 3·0–6·0 μ (3·5–6·5 μ with the verrucae). The species is readily separated from the remaining hygroscopic geasters, whose mouth characters are either sulcate or indeterminate.

Sowerby's validation of Woodward's *L. recolligens* appears to be the first specific name to be applied legitimately to this fungus. Although it seems that Woodward's species also included *G. triplex*, his most stressed characters are the strongly hygroscopic exoperidium and the silvery white fibrillose layer, neither of which are possessed by *G. triplex*. Sowerby's figure and specimen dispose of any doubts as to the fungus meant, hence his publication can be considered to have emended Woodward's species by confining it to the small, hygroscopic geaster with the determinate mouth. His specimen in Herb. Kew. is herewith designated as the Type.

G. mammosum Chev. and *G. corrollinum* Hollós are synonyms but *G. hungaricum* Hollós appears to be distinct, although the spore sizes which Staněk used for differentiating it from *G. recolligens* fall within the range of the type specimen as well as the world literature.

British records

G. recolligens appears to be rather uncommon in Britain, and it is open to question as to whether some records may not refer to *G. triplex*. Sandy banks appear to be a typical habitat. It has not yet been collected by the writer.

Woodward (1794) recorded it for Norfolk and Suffolk, whilst the Sowerby specimen was probably from Yorkshire. The fungus does not seem to have been found again until Berkeley & Broome (1876) reported that the

Rev. G. H. Sawyer had found it in Berkshire. The Cheshire record in Higgins (1859) was found by Palmer (1952) to refer to *G. triplex*.

Cooke (1937) listed a record by the Rev. Kirby Trimmer for Norfolk in 1861 and Mason & Grainger (1937) show it as having been found in two Yorkshire Vice-Counties.

The writer has examined a photograph of a freshly expanded geaster, determined as *G. mammosum* by Carleton Rea and collected by Mr R. H. Johnstone at Tynninghame, East Lothian, Scotland, in September 1934, which is very probably this species.

Spores and capillitia were measured in Erythrosin B in 10% ammonia (Palmer, 1955) and the figures were drawn freehand, with micro-characters measured by an ocular micrometer. The spore-size formulae show minimum, average and maximum measurements to the nearest 0·5 μ.

The author is grateful to Dr R. W. G. Dennis for his advice, to Mr R. H. Johnstone for details of the Scottish record, to Miss E. M. Wakefield for the loan of Bryant's rare pamphlet, and to the Director of the Royal Botanic Gardens, Kew, for facilities to examine the Sowerby specimen.

REFERENCES

Berkeley, M. J. & Broome, C. E. (1848). Notices of British fungi. *Ann. Mag. nat. Hist.* Ser. 2, **2**, 267–268.

Berkeley, M. J. & Broome, C. E. (1876). Notices of British fungi. *Ann. Mag. nat. Hist.* Ser. 4, **17**, 139.

Bryant, C. (1782). *An historical account of two species of* Lycoperdon. London. (Privately published.)

Chevalier, F. F. (1826). *Flore générale des environs de Paris*, **1**. Paris.

Coker, W. C. & Couch, J. N. (1928). *The Gasteromycetes of the Eastern United States and Canada*. Chapel Hill.

Cooke, G. J. (1937). Norfolk county list of fungi. *Trans. Norfolk Norw. Nat. Soc.* **14**, 292–300.

Fries, E. M. (1829). *Systema mycologicum*, **3**. Gryphiswald.

Gray, S. F. (1821). *A natural arrangement of British plants*, **1**. London.

Higgins, H. H. (1859). The fungi of Liverpool and its vicinity. Pt II. Gasteromycetes. *Proc. lit. phil. Soc. Lpool.* **13**, A, 123–138.

Hollós, L. (1904). *Die Gasteromyceten Ungarns*. Leipzig.

Lloyd, C. G. (1902). The Geastrae. *Bull. Lloyd Lib.* **5**, *Myc. Ser.*, no. 2.

Long, W. H. & Stouffer, D. J. (1948). Studies in the Gasteromycetes. XVI. The Geastraceae of the south-western United States. *Mycologia*, **40**, 547–585.

Mason, F. A. & Grainger, J. (1937). *A catalogue of Yorkshire fungi*. London.

Morgan, A. P. (1889). North American Fungi. The Gastromycetes. *J. Cincinn. Soc. nat. Hist.* **12**, 8–22.

Palmer, J. T. (1952). Lancashire and Cheshire earth-stars. *Proc. Lpool Nat. Fld Cl.* pp. 28–31.

Palmer, J. T. (1955). Observations on Gasteromycetes. 1–3. *Trans. Brit. mycol. Soc.* **38**, 317–334.

Persoon, C. H. (1801). *Synopsis methodica fungorum*. Göttingen.

Petri, L. (1909). Gasterales in *Flora Italica Cryptogama*, **1**.

Rea, C. (1922). *British Basidiomycetae*. Cambridge University Press.

Saccardo, P. A. (1888). *Sylloge fungorum*, **7**. Patavia.

Smith, W. G. (1873). Starry Puff Balls. *Gard. Chron.* p. 543.

Sowerby, J. (1809). *Coloured figures of English fungi or mushrooms*, **4**. London.

Staněk, V. J. (1953). Rehabilitace hvězdovky uherské—*Geastrum hungaricum* (Hollós, 1901) V. J. Staněk 1952 a její výskyt v ČSR. *Česká mykologie*, **7**, 34–42.

Woodward, T. J. (1794). An essay towards an history of the British stellated lycoperdons. *Trans. Linn. Soc.* **2**, 32–62.

(*Accepted for publication* 6 *July* 1956)

Trans. Brit. mycol. Soc. **40** (2), 283–291 (1957).

BEHAVIOUR OF SOIL FUNGI IN THE PRESENCE OF BACTERIAL ANTAGONISTS

By DAVID PARK

Department of Cryptogamic Botany, University of Manchester

In an attempt to differentiate between native soil fungi and alien fungi, selected organisms were compared in their reactions to bacterial antagonism. In agar and liquid cultures there was no distinction between the two categories. In sand and soil cultures containing a mixed bacterial flora, differences in behaviour were observed. In these artificial experimental situations, however, the distinctions were not between alien fungi and fungi native to a particular soil, but between soil inhabitants generally and fungi from other sources. The exochthonous fungi which remained viable in those cultures allowing of comparison, did so solely by means of inhibited spores, whereas the soil inhabitants spread through the substratum as a mycelium, some cells of which persisted throughout the period of observation.

Introduction

Problems relating to the establishment, growth and survival of fungi in a soil have already been investigated by the author, by comparing the behaviour of fungi isolated from that soil with that of alien fungi (Park, 1955). The results indicated that differences between the two categories resolved into (*a*) ability to colonize successfully solid decomposable materials in the soil, and (*b*) ability to survive in soil in such materials. These differences were expressed in the presence of other organisms, i.e. under conditions of antagonism. The present paper describes the reactions of fungi, under various cultural conditions, to the presence of antagonistic bacteria obtained from that soil from which the native fungi were isolated.

Materials and methods

The twelve fungi used in these experiments, and their sources, were:

Natives

Mucor silvaticus *Fusarium roseum* *Monotospora daleae* *Penicillium roqueforti* *Cladosporium cladosporioides* *Trichoderma viride*	From native soil, September 1953

Aliens

Rhizopus sexualis	From decaying strawberry, 1922
Aspergillus niger	Aerial contaminant, 1949
Botrytis cinerea	From diseased *Fuchsia* stem, 1949
Penicillium digitatum	From decaying lemon fruit, 1949
Stemphylium sarcinaeforme	From an 'alien' soil, November 1952
Trichothecium roseum	From decaying apple fruit, 1945

The antagonists were, in one series, *Bacillus macerans*, and, in the other, the organisms growing from propylene oxide-treated soil. Soil and sand cultures were made in Petri dishes, the sand-culture experiments having duplicate series with Czapek's solution and soil solution as the nutrient liquids. Liquid cultures, again with Czapek's solution and soil solution, contained 40 ml. medium in 100 ml. flasks. Agar cultures were made on Czapek's medium.

Microscopic observations of the developments in sand and soil cultures were made from contact slides and smear slides. Determinations of viability in these cultures were made by Warcup soil plates, using rose bengal agar. Microscopic observations from liquid and agar cultures were made by culture mounts in lactophenol/acid fuchsin. Details of these materials and methods are given in previous papers (Park, 1955, 1956).

Survival of alien species in fresh soil

In a previous study (Park, 1955), spores of alien species added to fresh soil were afterwards not detectable by the contact slide method. However, since Park (1956) has shown that a fungus may be viable in a substratum although its conidia no longer appear on contact slides, it was thought desirable to determine as critically as possible, (i) the ability of alien species to survive in fresh soil in the laboratory, (ii) the reliability of the contact slide method for this sort of observation. To this end three methods of observation were used, namely, contact slides, smear slides, and Warcup soil plates. The slide areas of soil dishes prepared from fresh soil were inoculated separately with spores of the six alien fungi. Observation by the three methods was continued for 16 weeks; the maximum survival time for each fungus as indicated by each method is shown in Table 1.

Table 1. *Survival of alien organisms added to fresh soil, as determined by different methods*

(Survival time in weeks.)

	Contact slide	Smear slide	Soil plate
Rhizopus sexualis	0	0	0
Aspergillus niger	1	7	7
Botrytis cinerea	1	4	5
Penicillium digitatum	2	6	6
Stemphylium sarcinaeforme	2	16	16
Trichothecium roseum	1	3	2

These results confirm that the contact slide method gives an erroneous estimate of survival time; closer agreement occurs between the results from the other two methods. Within the sensitivity of the methods, the alien fungi, with the exception of *Stemphylium sarcinaeforme*, are eliminated from the population of fresh soil within the period of observation. No germination of any alien spores was observed; although the usual small number of short hyphal segments was seen, they could not be assigned with certainty to any of the alien fungi.

Bacillus macerans as antagonist to the fungi

On agar

The bacterium and the appropriate fungus were inoculated as discrete colonies at opposite sides of Petri dishes. No clear-cut distinction could be drawn between the reactions of the two categories of fungi to the bacterial colony. Various types of behaviour were induced in the opposed fungus, namely, continuous but reduced growth towards the bacterial colony; a sharp check to the mass of hyphal tips, followed by production of pioneer hyphae; chlamydospore formation; lysis of some of the hyphae entering the bacterial colony, etc. This variety of reactions was found in both alien and native fungi. *Rhizopus sexualis* was the only fungus of which hyphae did not eventually enter the bacterial colony.

In liquid culture

Flasks containing the nutrient solutions were simultaneously inoculated with the two antagonists. Observations were made for 21 days. Growth of the various fungi in the presence of the bacterium was slower than that in its absence, and the hyphae in contact with the liquid were commonly coated with a sheath of bacterial cells; hyphal distortions and lysis were common in fungi of both classes. In neither solution were general differences in reactions obtained between the two categories of fungi. All twelve fungi, with the exception of *R. sexualis* in Czapek's solution, maintained some hyphae of normal form, retained viability, and continued growing.

In sand culture

The behaviour in sand cultures inoculated with the antagonists in pairs was observed from smear slides and soil plates over a period of 16 weeks. With the exception of *R. sexualis*, which was eliminated from the cultures after 3 weeks, the fungi, although subject to lysis and antagonized by the bacterium, remained viable.* No differences in the reactions of the two categories of fungi could be detected: all maintained a small amount of mycelium as short hyphal segments, but were present largely as resistant spores. The conidia of *Monotospora daleae*, *Botrytis cinerea*, *Stemphylium sarcinaeforme* and *Trichothecium roseum* typically had thick walls. The asexual spores of the other species, in the cultures from which the inocula were taken, had thin walls. After the addition of these thin-walled spores to the sand cultures containing the bacterium it was found that, with the exception of those of *R. sexualis*, an increase in the thickness of the wall took

* In this experiment, in the Czapek's solution series one culture inoculated with *Botrytis cinerea* was found contaminated with *Trichoderma viride* at the 5-week stage. At the 6-week stage *B. cinerea* was no longer viable in this culture. A dish of *Penicillium roqueforti* also became infected with the same organism at the 3-week stage: the presence of the contaminant here did not affect the viability of *P. roqueforti*. Similarly, in the soil solution series one dish of *B. cinerea*, two of *P. digitatum* and one of *T. viride* became contaminated with an unidentified species of *Penicillium*. *B. cinerea* and *P. digitatum* disappeared from their cultures, whereas *T. viride* remained viable in the presence of the contaminating organism.

place. In *T. roseum* no increase in the thickness of the wall was detected, the only change in the spores being a tendency for the proximal cell to be decomposed, while the remaining cell retained viability, appearing as a single-celled spore. In *B. cinerea* and *M. daleae* the thick wall already present appeared to become erose in the presence of the antagonist, and later preparations gave the impression that the thickness of the wall had been increased from within. The walls of the dark-coloured *Stemphylium* conidia are difficult to observe but photographs show them as originally thick. After 6 weeks in bacterial culture in sand, the cells of the spores of this species had swollen in a manner reminiscent of those of *Fusarium* conidia in soil and sand culture (Park, 1956). Here, as in that case, conidia not undergoing this development became devoid of contents.

The changes which occurred in these inhibited spores, namely increase in cell size, increase in wall thickness, and increase in density of protoplasm, indicate conversion of a vegetative cell into a chlamydospore. It is generally accepted that one or more cells of a *Fusarium* macroconidium may become chlamydosporic by this sort of change. The changes in the unicellular asexual spores are similarly interpreted, the process being regarded as an adaptation of the spore to conditions unfavourable for germination and growth.

In addition to these resting spores from modified conidia or sporangiospores, mycelial chlamydospores were produced in *Mucor silvaticus*, *Fusarium roseum*, *Cladosporium cladosporioides*, *Trichoderma viride* and *Stemphylium sarcinaeforme*. Chains of fumagoid cells were also produced by *C. cladosporioides*; these are akin to chlamydospores (Langeron, 1952), as are the 'conidia' (aleuriospores) of *Monotospora daleae* (Mason, 1941). Mycelial chlamydospores have an advantage not possessed by thick-walled conidia and sporangiospores—they can be produced rapidly from the active vegetative body of the fungus without the prior formation of spore-bearing, specialized hyphae.

In soil culture

Observations in autoclaved soil dishes inoculated with the antagonists were made by smear slides and by soil platings over a period of 16 weeks. As in sand culture all fungi germinated and grew, and all, except *R. sexualis*, remained viable throughout the experiment, mainly by means of the resistant spores produced. A low level of mycelium could be detected throughout the experiment in the cultures of nine fungi, the exceptions being *B. cinerea*, *Trichothecium roseum* and *R. sexualis*. The last-named fungus was non-viable after 10 days.

Organisms from propylene oxide-treated soil as antagonists to the fungi

In these experiments fresh soil which had been exposed to propylene oxide vapour for 24 hr. and incubated thereafter for 14 days was used as the source of the antagonists. Agar, liquid and sand cultures were inoculated with small crumbs of the soil. For the soil cultures, soil dishes which had been exposed to the antiseptic vapour and incubated were themselves used for the cultures.

On agar

The behaviour in the early stages of the experiment, i.e. prior to contact of the organisms, was of the same type as that described for the *Bacillus macerans* antagonism. After contact of the fungus with the bacterial mass, a difference was apparent in that here components of the bacterial flora spread actively at an increased rate into the fungal colonies, causing lysis of some of the structures present. No general differentiation could be made between the reaction of the native and alien fungi to this invasion. Lysis of hyphae and of spores of all types was seen, but the fungi, with the exception of *R. sexualis*, retained some viable spores, and some active mycelium. Growth and sporulation of the eleven fungi continued. After 3 weeks the plates were beginning to become dry, and were flooded on the surface with sterile water. After this treatment the bacteria developed more vigorously than before, but no change in the relationships was observed. An equilibrium appeared to have been reached, and the eleven fungi continued to grow and sporulate at the same time that they were being lysed.

In liquid culture

The behaviour of the fungi did not differ from that described under the corresponding section in the presence of *B. macerans*. Lysis occurred but was less extensive than that in the agar cultures just described. Again it was not possible to differentiate the two categories of fungi on the basis of this experiment.

In sand culture

All the fungi germinated and produced mycelium, which in most species sporulated, but after several days lysis of mycelium and spores was seen, and rapidly became extensive. *R. sexualis* was eliminated from both series within 10 days. The remainder of the fungi survived throughout the 16 weeks, so that no differentiation of categories could be made on this survival basis. However, all the native species maintained some viable mycelium throughout the 16 weeks. The only alien to do this was *Stemphylium sarcinaeforme*, the others retaining viability by inhibited resting spores only.

In soil

The added sporangiospores of *R. sexualis* remained inactive, and all were digested in the first 10 days; the other eleven fungi remained viable throughout the 16-week observation period. Germination of the added spores of native fungi took place, the resulting sporulating mycelium becoming subject to lysis. These species, however, retained a small amount of mycelium, as well as inactive spores, throughout the experiment. *S. sarcinaeforme* behaved in the same manner. The added spores of the other four alien species were not seen to germinate, and at no time was mycelium found in the cultures of these organisms, which survived solely by means of the added spores.

Spread of fungi in soil treated with propylene oxide vapour

In the previous experiments the fungi of the two categories could, with one exception, be differentiated on the presence or absence of mycelium over a long period in propylene oxide-treated soil. Hyphae, where present, might enable the possessor to make persistent growth through the substratum, even though a spatially continuous mycelium were not present.

Petri dishes containing soil that had been exposed to propylene oxide vapour for 24 hr., and then incubated for 14 days, were each inoculated locally near one side with 0·2 ml. of a dense suspension of spores of the appropriate test fungus. The inoculum was put down as a small drop, the area of which was marked on the lid of the dish. Weekly determinations of fungal spread were made by taking samples of soil at centimetre intervals from the edge of the inoculated area, and plating on rose bengal agar (Table 2).

Table 2. *Spread of fungi through propylene oxide-treated soil*

(Growth in cm. at weekly observations.)

Weeks	1	2	3	4	5	6	8
Mucor silvaticus	0	0	1	2	2	3	3
Fusarium roseum	0	1	3	4	7	*	*
Cladosporium cladosporioides	0	0	0	0	1	2	2
Monotospora daleae	0	0	1	3	7	*	*
Penicillium roqueforti	0	0	0	1	1	1	2
Trichoderma viride	0	0	1	1	2	3	5
Rhizopus sexualis	0	0	0	0	0	0	0
Aspergillus niger	0	0	0	0	0	0	0
Botrytis cinerea	0	0	0	0	0	0	0
Penicillium digitatum	0	0	0	0	0	0	0
Stemphylium sarcinaeforme	0	0	0	0	1	1	2
Trichothecium roseum	0	0	0	0	0	0	0

* Dish filled, i.e. growth greater than 7·5 cm.

It is clear from Table 2 that measurable growth had been made only by those fungi that had maintained some mycelium in sand and soil in the presence of the organisms growing from propylene oxide-treated soil.

Control series of pure cultures of fungi in sand and soil

Dishes of autoclaved soil, and autoclaved sand cultures with Czapek's solution and with soil solution were individually inoculated with separate fungi. Observations, over 16 weeks, were made by smear slides and soil plates.

In all substrata each fungus gave an initially vigorous growth and sporulation. The activity was greatest in sand + Czapek's solution, and least in sand + soil solution. After 3–4 weeks, some autolysis of structures present was seen. *R. sexualis* had become non-viable after 5 weeks in the sand cultures, and after 11 weeks in the soil cultures. The other eleven species retained a moderately high proportion of sporulating mycelium on all substrata; this amount of mycelium, although less than that at first

occurring, was higher than that found in the experiments where bacterial antagonists inhabited the sand and soil substrata.

The author has previously (1956) suggested that heterolysis and autolysis need not be mutually exclusive processes. In fact, the phenomena of lysis and inhibition of fungi, observed in sand and soil cultures in the presence of bacteria, were an intensification of the phenomena observed here in old pure cultures on these substrata. The fungi in these old pure cultures also exhibited features characteristic of antagonism as found in natural soil. Thus antagonism of fungi in nature exhibits features similar to those characterizing 'staling' in pure cultures. This fact is not new, but its significance has become obscured in much of the discussion about the role played in nature by specific antibiotics.

Discussion

The author's studies are essentially directed towards determining whether any inferences or conclusions of a general nature relating to the biology and ecology of soil fungi can be stated. It is apparent that since the soils which might be investigated are almost endlessly varied, it is, in practice, only feasible to investigate small numbers of selected soils or micro-organisms. The writer has, therefore, attempted to compare the behaviour of fungal isolates native to a particular soil with isolates alien to it, in the hope that information on the attributes affecting the ability of fungi to inhabit soil may be obtained. It was from this point of view, namely, in relation to the ecology of a particular soil, that the categories 'native soil fungi' and 'alien fungi' were defined (Park, 1955).

A difference in behaviour between the native and alien fungi in the present experiments had been anticipated by the author. However, in those experiments which exhibited contrasts, the alien fungus, *Stemphylium sarcinaeforme*, differed from the other alien species and behaved like the native species. This fungus had, in fact, been isolated from a soil different from that used as the basis for the classification into native and alien species, so that, although alien to that soil, the fungus was a soil inhabitant. The other five fungi classified as aliens were obtained from other habitats; although the possibility that these isolates were soil inhabitants cannot be absolutely excluded, their habitats indicate a lower degree of antagonism than that present in soil.

The result anticipated, that of a distinction between the native and alien fungi, was based on the hypothesis that the native fungi are more tolerant than the alien fungi to those antagonistic conditions found in the 'native' soil. This hypothesis is still tenable: the reasoning did, however, fail on the implicit second premise, that the antagonistic conditions provided in these experiments are more closely comparable to the conditions found in the 'native' soil than to those found in soils more generally. There are no good grounds for believing this to be so. Consequently, if the antagonistic conditions provided in these experiments may be taken to represent types of antagonistic conditions found in soils generally, the results of the study should be made applicable to soil fungi as a whole, and the comparison made between soil inhabitants and fungi from other sources. It is appropriate

to note that, in this wide sense, the antithesis of soil inhabitants is not soil invaders (Reinking & Manns, 1933), but all other fungi. Such organisms may be qualified by the adjective 'exochthonous'. *Exochthonous fungi* are defined as *fungi of which the habitual substrata are not regarded as part of the soil, and which do not maintain themselves in an active condition in soil.* The derivation of the word 'exochthonous' was suggested by comparison with 'autochthonous' (Winogradsky, 1924), but it should be noted that the two terms as defined are not direct contrasts, an autochthonous flora existing in 'normal' or 'resting' soil, and being a part of the soil inhabiting flora. Garrett (1950) gives a schematic representation of the terminology used for different groups of root-infecting fungi. A scheme of this type for soil fungi generally, which may help to clarify their ecological relationships, is:

Autochthonous fungi, Zymogenous fungi	Soil inhabiting fungi	Adapted to continued vegetative activity in the soil substratum
Exochthonous fungi	Soil invading fungi; 'Non-soil' fungi	Ill-adapted to continued vegetative activity in the soil substratum

As in other biological classifications, the groups are not necessarily sharply delimited from each other. The fungi used in the present studies are considered to comprise seven soil-inhabiting species (six natives and one alien) and five exochthonous species (five aliens).

In a previous paper (Park, 1955) three attributes of probable importance to fungi in the soil habitat were considered. One of these dealt with the ability of fungi to make vegetative mycelial growth through the soil, and it was found that in this respect no contrast could be made between the two categories of fungi. However, in the more severe experimental situations in the present study, the soil inhabitants were able to make a continuous spread through the substratum, whereas the exochthonous fungi were not. This spread of the soil inhabitants was slow, and was characterized by the presence of short hyphal segments separated from each other by digestion of intermediate cells. Such a method of spread, if it is of general occurrence, may be important in the biology of fungi in soil, in that it enables them to increase their population level and their chance of coming into contact with suitable substrata.

Another attribute considered previously, namely, ability to survive inimical conditions, was investigated here with regard to bacterial antagonism. In this respect there was no general difference between the two categories of fungi. In fact, only under the conditions of accidental fungal contamination of the cultures did certain of the exochthonous fungi become non-viable. The relative sensitivity of soil inhabitants and of other fungi to fungal antagonisms will form the subject of a separate paper.

The author is much indebted to Prof. C. W. Wardlaw and to Dr S. D. Garrett for discussion and criticism during the preparation of the manuscript.

REFERENCES

GARRETT, S. D. (1950). Ecology of root-inhabiting fungi. *Biol. Rev.* **25**, 220–254.
LANGERON, M. (1952). *Précis de mycologie.* Paris.
MASON, E. W. (1941). *Annotated account of fungi received at the Imperial Mycological Institute. List II. Fasc.* 3. *Special part*, pp. 101–144. (I.M.I., Kew.)
PARK, D. (1955). Experimental studies on the ecology of fungi in soil. *Trans. Brit. mycol. Soc.* **38**, 130–142.
PARK, D. (1956). Effect of substrate on a microbial antagonism, with reference to soil conditions. *Trans. Brit. mycol. Soc.* **39**, 239–259.
REINKING, O. A. & MANNS, M. M. (1933). Parasitic and other Fusaria counted in tropical soils. *Z. Parasitenk.* **6**, 23–75.
WINOGRADSKY, S. (1924). Sur la microflore autochthone de la terre arable. *C.R. Acad. Sci., Paris*, **178**, 1236–1239.

(*Accepted for publication* 8 *June* 1956)

Trans. Brit. mycol. Soc. **40** (2), 292–294 (1957).

ON THE MARINE PHYCOMYCETE *THRAUSTOCHYTRIUM PROLIFERUM*

By T. W. JOHNSON, Jr.

Department of Botany, Duke University, Durham, North Carolina, U.S.A.

(With 1 Text-figure)

Resting spores, planont cleavage, flagellation, and sporangial development are described for a variant of *Thraustochytrium proliferum* Sparrow.

The characteristics of *Thraustochytrium proliferum*, in so far as they were known at the time, were given by Sparrow (1936) who later, (Sparrow, 1943) described planont flagellation. In both descriptions the two principal generic characteristics, thraustothecoid planont discharge and internal sporangial proliferation, were stressed. Among the characteristics of this marine fungus not recorded are those of the resting spores and the mode of planont cleavage.

In May 1955, and again in 1956, *T. proliferum* was found on decaying *Bryopsis plumosa* (Hudson) C. Agardh in the Beaufort Inlet, Beaufort, North Carolina. The fungus was sufficiently abundant for a detailed study of its development. This paper is a report on these observations.

The characteristics of the Beaufort material differ somewhat from those previously recorded. Although planont size is the same as that originally described (Sparrow, 1943), the sporangia are considerably larger: 22–29 μ high by 16–20 μ in diameter. Furthermore, the rhizoidal system is generally more profusely developed (Fig. 1 *u*) than in Sparrow's collection. Sparrow described the sporangia as pyriform (Fig. 1 *o*, *p*, *w*); in the Beaufort material these are clavate (Fig. 1 *z*), asymmetrical (Fig. 1 *n*), and nearly globose (Fig. 1 *q*) as well.

The initial development features of *T. proliferum* are very similar to those of *Chytridium* (Sparrow, 1943, p. 25). Motile planonts come to rest on the substratum and produce a short, intramatrical, peg-like rhizoid fundament which soon branches (Fig. 1 *a*). The planont then enlarges into the primary sporangial initial (Fig. 1 *b*, *c*). Prior to initiation of spore cleavage the sporangial protoplasm becomes densely vacuolate. Cleavage begins at the apex of the sporangium (Fig. 1 *d*) and rapidly proceeds basi-petalously (Fig. 1 *e*). The secondary sporangial initial is cleaved during the formation of the basal spore initials (Fig. 1 *e*). Thus, the new sporangial fundament is formed before the spores are discharged, as Sparrow suggested.

Two factors are contributory to spore discharge: swelling of the spore initials, and enlargement of the secondary sporangium initial (Fig. 1 *f*, *g*). The entire sporangium increases in size as a result of these enlargements (Fig. 1 *g*). Spore discharge is characteristically thraustothecoid, that is, the

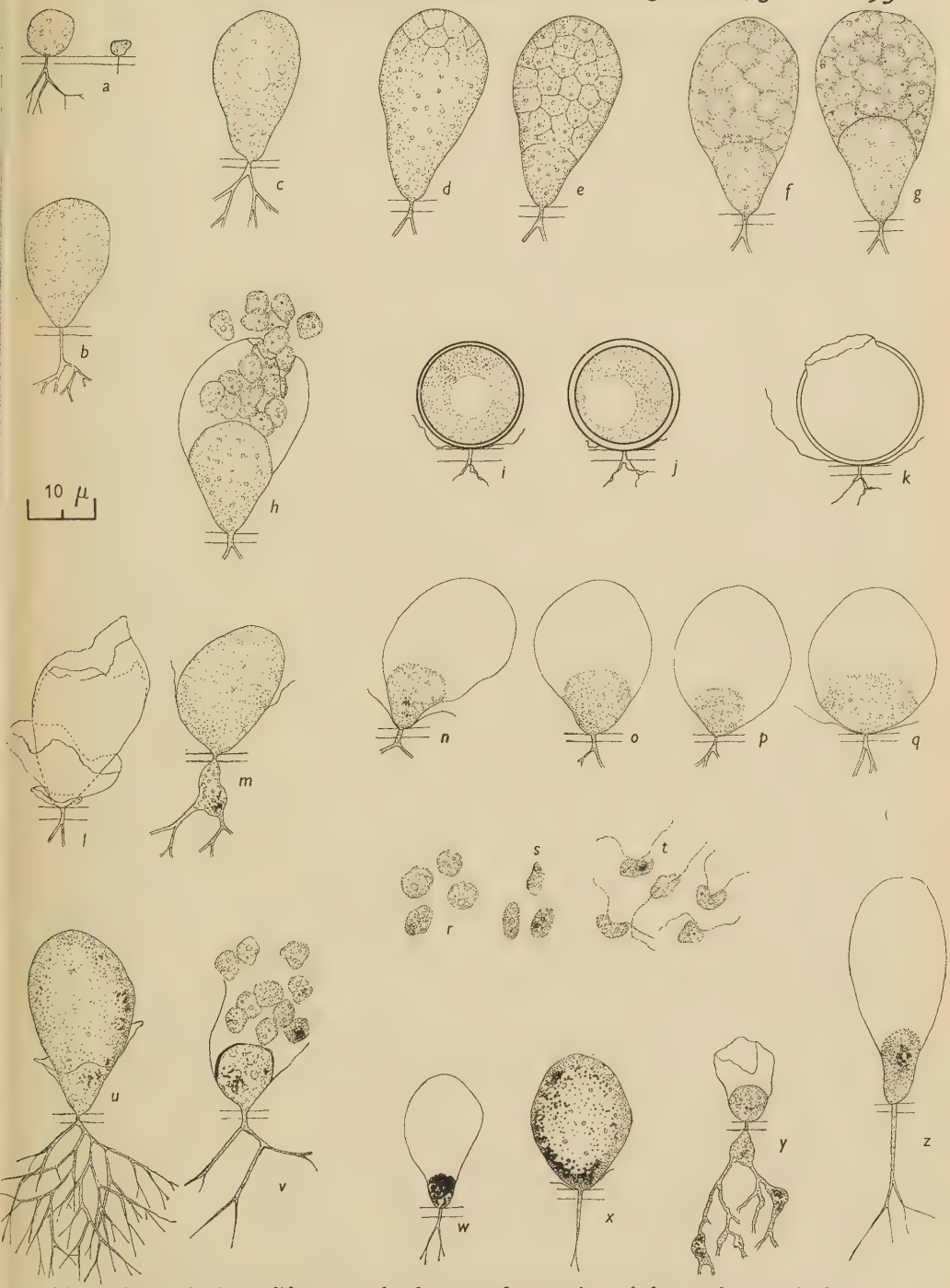


Fig. 1. *Thraustochytrium proliferum.* *a–g*, development of sporangia, and planont cleavage; *h*, planont discharge; *i–k*, resting spores; *l*, sporangial proliferation; *m*, proliferating sporangium with subsporangial apophysis; *n–q*, *w*, *x*, *z*, variations in sporangial shape; *r–t*, planonts; *u*, *v*, two sporangia showing variations in extent of rhizoidal system; *y*, small sporangium with ellipsoid swellings in the intramatrical rhizoid system.

sporangium wall bursts or deliquesces and the spores are liberated as non-motile cells (Fig. 1 *h*). The discharged spores remain for some time in a loose aggregation at the irregular sporangial orifice (Fig. 1 *v*). At the time of discharge the spores are aplanetic, and are usually angular (Fig. 1 *r*) with one vacuole and a small refractive globule. The first evidence of motility is observed as a change in shape from angular to ellipsoid (Fig. 1 *s*). The spore then rapidly assumes a reniform or gibbose shape, and two flagella appear (Fig. 1 *t*). The flagella are laterally attached, but because of the slight amoeboid motion they often appear apically attached. The type of planont flagellation is further evidence that *T. proliferum* is closely related to members of the genera *Thraustotheca* and *Brevilegnia* (Saprolegniaceae) in which the spores emerge from cysts as laterally biflagellate planonts. There is no direct evidence of a primary encystment of the spores in *T. proliferum*, but since the spores are non-motile at discharge, they are very likely in a primitive type of encystment stage. No cyst wall is left, however, as the spores assume motility. Repeated internal proliferation often follows each successive spore discharge, with the preceding sporangial walls usually being left in evidence (Fig. 1 *l*).

The resting spores of *T. proliferum* are spherical, smooth, and 13–19 μ in diameter. They apparently develop from the same rhizoidal system that anchors the sporangia since a portion of the sporangial wall is always detectable at the base of the resting spore (Fig. 1 *i–k*). The resting spore wall shows a reaction of unmodified cellulose when treated with chloroiodide of zinc. Internally, the cytoplasm has a single, centric or eccentric globule (Fig. 1 *j*, *k*). Germination or discharge of these resting spores was not observed. Two empty spores, each exhibiting a large, slightly irregular orifice (Fig. 1 *k*) were seen, however.

The rhizoidal system is remarkably variable in extent. A much-branched, profuse system is usually formed (Fig. 1 *u*), but a single, peg-like and once-branched rhizoid may also develop (Fig. 1 *x*). Such a reduced rhizoid suggests a similar condition in *Thraustochytrium globosum* (Kobayashi & Ookubo, 1953). Occasionally the rhizoids are marked by ellipsoid or irregularly-ellipsoid swellings (Fig. 1 *y*), and in infrequent instances a sub-sporangial apophysis occurs in the rhizoid immediately below the host wall (Fig. 1 *m*). The latter condition suggests the rhizoidal system reported for *Japonochytrium marinum* (Kobayashi & Ookubo, 1953).

This investigation was supported by funds from the National Science Foundation and the Duke University Council on Research. I am grateful to Prof. J. S. Karling for evaluation and editing of the manuscript.

REFERENCES

Kobayashi, Y. & Ookubo, M. (1953). Studies on the marine Phycomycetes. *Bull. nat. Sci. Mus., Tokyo*, **33**, 53–65.

Sparrow, F. K., Jr. (1936). Biological observations on the marine fungi of Woods Hole waters. *Biol. Bull., Woods Hole*, **70**, 236–263.

Sparrow, F. K., Jr. (1943). *Aquatic phycomycetes*. Ann Arbor: University of Michigan Press.

(*Accepted for publication* 17 *August* 1956)

PROCEEDINGS

THE SHEFFIELD FORAY

6 TO 12 SEPTEMBER 1956

BY S. M. FRANCIS, *Foray Secretary*

The fifty-second Autumn Foray was held at Sheffield from 6 to 12 September. We were accommodated in the University Hall for women, where the Warden and her staff made us extremely comfortable. An excellent workroom and a display room were made available in a near-by house. Our thanks are due to Prof. Clapham for the loan of microscopes and binoculars from his department.

We were fortunate in having Dr Emil Müller from Zürich as our distinguished guest. Dr Müller, who is well known for his contributions to the taxonomy of the Pyrenomycetes, very kindly presented a collection of Alpine Pyrenomycetes to the Society (see p. 300).

The Foray was attended by about thirty members—a smaller number than usual. The European Mycological Congress held in Belgium the week after our Foray attracted about twenty members, few of whom could afford either the time or money to attend both meetings.

At a dinner on Friday night we were delighted to welcome Mrs Astley-Cooper, the daughter of the late Carleton Rea. We were also pleased to have with us Prof. Clapham and Mr A. C. Collinge (the Chairman) and Miss J. Grainger (Secretary of the Mycological Committee of the Yorkshire Naturalists' Union).

There were three full-day excursions, Clumber Park on Friday, 7 September; Millersdale and Cressbrook on Monday, 10 September (this was instead of the advertised excursion to Roche Abbey); and, on Tuesday, 11 September we visited Chatsworth House where members spent some time in looking round the famous gardens. As half-day excursions we visited Longshaw Pastures on Saturday, 8 September, where sheep dog trials provided a counter attraction to fungus hunting, and on the Sunday afternoon we had a long and enjoyable walk to Porter Clough, Ringinglow and Whirlow.

Our collection of agarics was only just over the 200 mark, thus falling short of Dr Hora's estimate that an average Autumn Foray should yield 250. I have to report, therefore, that as far as agarics went, the foray was slightly below average. Dr Hora tells me that a new find was *Hygrophorus brevispora*, first described by Möller from The Faroes in 1945. Other species which have not yet appeared in our lists, but which have been found on previous occasions are *Hygrophorus aurantio-splendens*, *H. subminutulus*, *Coprinus xanthothrix* and *Pluteus hiatulus*. Very fine specimens of the quite uncommon *Amanitopsis inaurata* were also collected.

In the Discomycete section, our President claims that 'preoccupations of office' kept him from collecting as zealously as usual. Two uncommon finds were *Helotium laetum* and *Trichophaea woolhopeia*, while *Peziza aurantia* in a superb cluster some 6 in. across was most spectacular.

The highlight of the Pyrenomycete list was a collection by our visitor, Dr Müller, of *Melanospora theleboloides*. This appears to be a new British record; material has been deposited in the herbarium at Sheffield.

A collection of his new species *Nidularia minutissima* is reported by Mr Palmer (see *Naturalist, Lond.*, 1957, Jan./Mar.).

The rust list is very meagre, and Dr Manners reports that he has never found fewer species on a Foray, although several of the places visited were obviously good rust habitats.

Foray arrangements for 1957 were discussed at a business meeting held on Monday evening. We received an invitation to visit Dublin from Dr O'Connor and, as a possible alternative, Aberystwyth was suggested. A vote was taken and the majority favoured Dublin.

I would like to thank Dr Webster for all the hard work he put into organizing this Foray, also Dr Brown, Mr Graddon, Dr Hora, Prof. Ingold, Dr Manners, Mr Orton and Mr Palmer for the help they have given me in compiling the List of Species.

I am especially grateful to Mrs Holman and Miss Kennedy who noted the names of agarics as Dr Hora and Mr Orton identified them in the field, and helped in arranging the specimens on the display tables.

List of species

Ch. = Chatsworth House; *Cl.* = Clumber Park; *H.* = University Hall; *L.* = Longshaw Pastures; *M.* = Millersdale and Cressbrook; *R.* = Porter Clough, Ringinglow and Whirlow. Voucher material: [1] = in herb. J. T. Palmer; [2] = in herb. University of Sheffield. † = a new British record.

For nomenclature see these *Transactions*, **38**, 185 (1955).

MYXOMYCETES

Arcyria cinerea; denudata, *Cl.*; pomiformis.
Comatricha nigra.
Craterium minutum.
Fuligo septica.
Hemitrichia abietina, *Cl.*
Lamproderma violaceum.
Leocarpus fragilis.
Lycogala epidendrum.
Physarum sinuosum; viride var. incanum.
Stemonitis flavogenita.
Trichia scabra, *Cl.*; varia, *Cl.*, *M.*
Tubifera ferruginosa.

ASCOMYCETES

Pyrenomycetes

Daldinia concentrica, *R.*
Diatrype disciformis, *Ch.*, *Cl.*; stigma, *Cl.*
Eutypa flavovirens, *Cl.*
Hypocrea rufa, *L.*
Hypoxylon coccineum, *Ch.*; rubiginosum, on *Fagus*, *Cl.*
Lasiosphaeria ovina, *Cl.*

Lophiosphaera fuckelii f. rubi.
†[2]Melanospora theleboloides (Fuckel) Winter, host undetermined, *Cl.*
Nectria sinopica, *Cl.*
Stigmatea robertiana.
Xylaria hypoxylon, *Ch.*, *Cl.*; polymorpha, *Ch.*

DISCOMYCETES

Anthracobia melaloma, *Cl.*
Ascobolus denudatus, *Cl.*; stercorarius, *Cl.*
Cheilymenia coprinaria, *Cl.*; dalmeniensis, *Cl.*
Chlorociboria aeruginosa (Fr.) Seaver.
Coryne sarcoides, *Cl.*
Dasyscypha nudipes, *Cl.*
Helotium fagineum (Fr.) Fr., *L.*; fructigenum, *L.*; laetum (Boud.) Sacc., *L.*
Helvella crispa, *L.*; lacunosa, *Cl.*
Humaria roumegueri var. carnosissima, *L.*
Hyaloscypha deparcula (Karst.) Nannf. *Cl.*; stevensoni (Berk. & Br.) Nannf., *L.*
Lachnea hemispherica, *Cl.*
Lasiobolus equinus, *Cl.*
Leotia lubrica, *Ch.*
Macropodia macropus, *Cl.*
Mollisia cinerea, *Cl.*; discolor, *Cl.*
Otidea leporina, *Cl.*; onotica, *Cl.*
Peziza aurantia, *Cl.*
Phaeobulgaria inquinans (Fr.) Nannf., *Cl.*
Pseudopeziza trifolii.
Rhizina inflata, *L.*
Rutstroemia echinophila (Fr.) v. Höhn., *Cl.*; sydowiana (Rehm) White, *Cl.*
Scutellinia scutellata (Fr.) Lamb, *L.*; vitellina (Fr.) Lamb, *L.*
Trichophaea woolhopeia, *L.*

HETEROBASIDIOMYCETES

TREMELLALES

Auricularia auricula-judae, *Cl.*; mesenterica, *Ch.*
Calocera viscosa, *Ch.*, *Cl.*, *L.*, *R.*
Dacryomyces deliquescens, *Ch.*, *Cl.*, *L.*

UREDINALES

Coleosporium petasitis, on *Petasites hybridus*, *M.*; tussilaginis, on *Tussilago farfara*, *M.*
Melampsora tremulae, on *Populus tremula*, *L.*
Melampsoridium betulinum, on *Betula pubescens*, *L.*
Puccinia airae, on *Deschampsia caespitosa*, *Cl.*; arrhenatheri, on *Arrhenatherum elatius*, *R.*; chaerophylli on *Myrrhis odorata*, *R.*; poarum, on *Tussilago farfara*, *M.*

HOMOBASIDIOMYCETES—HYMENOMYCETES

POLYPORALES

Clavaria cinerea, *Cl.*; corniculata, *Ch.*; fumosa, *Ch.*; inaequalis, *Cl.*; kunzei, *Ch.*; rugosa, *R.*
Fistulina hepatica, *Cl.*
Fomes fomentarius, *Cl.*
Ganoderma applanatum, *Ch.*
Hydnum repandum, *R.*
Polyporus adustus, *Cl.*; betulinus, *Ch.*, *Cl.*, *L.*, *R.*; giganteus, *Ch.*, *Cl.*; lacteus, *Cl.*; schweinitzii, *Cl.*
Polystictus versicolor, *Ch.*, *Cl.*, *M.*
Poria mucida, *Cl.*
Stereum gausapatum, *Cl.*; hirsutum, *Ch.*, *Cl.*; purpureum, *Ch.*, *Cl.*
Thelephora terrestris, *Ch.*, *Cl.*

AGARICALES

Amanita citrina, *Ch.*, *Cl.*; excelsa, *R.*; muscaria, *Cl.*; rubescens, *Ch.*, *Cl.*, *L.*, *R.*
Amanitopsis fulva, *L.*, *R.*; inaurata, *Ch.*, *L.*; vaginata, *R.*
Anellaria semi-ovata, *L.*
Armillaria mellea, *Ch.*, *Cl.*, *M.*, *R.*
Boletus badius, *Ch.*, *Cl.*; chrysenteron, *Ch.*, *Cl.*, *R.*; edulis, *Cl.*, *R.*; piperatus, *Cl.*; scaber, *Cl.*; subtomentosus, *L.*; versicolor, *Ch.*, *Cl.*, *R.*; versipellis, *Ch.*, *Cl.*
Cantharellus cibarius, *L.*
Clitocybe candicans, *Cl.*; clavipes, *Cl.*, *R.*; ditopus, *R.*; flaccida, *Cl.*; gigantea, *Ch.*; infundibuliformis, *Cl.*; rivulosa, *Ch.*; suaveolens, *M.*
Collybia ambusta, *Cl.*; butyracea, *Cl.*; cirrhata var. cookei, *R.*; maculata, *Ch.*, *Cl.*, *R.*; ozes sensu Lange, *Cl.*; platyphylla, *Cl.*; radicata, *Ch.*, *Cl.*, *L.*, *R.*; velutipes, *Ch.*
Coprinus atramentarius, *Ch.*, *M.*; comatus, *Ch.*, *Cl.*; lagopides, *Cl.*; micaceus, *Ch.*, *Cl.*, *M.*; plicatilis, *Cl.*, *M.*; radians, *Ch.*; xanthothrix Romagn., *Cl.*
Cortinarius causticus, *R.*; cinnamomeus agg., *Cl.*; duracinus, *R.*; largus, *R.*; scandens, *Cl.*
Craterellus cornucopioides, *Ch.*
Crepidotus luteolus Lamb, *M.*; variabilis, *Ch.*
Crinipellis stipitarius, *Cl.*
Eccilia polita sensu Lange, *Cl.*; undata, *Cl.*
Entoloma porphyrophaeum, *Ch.*
Flammula alnicola, *R.*; carbonaria, *Cl.*; gummosa, *Ch.*; penetrans, *Ch.*, *Cl.*, *R.*; sapinea, *Cl.*
Galera appendiculata, *Cl.*, *R.*; clavata, *M.*; hypnorum, *Ch.*, *Cl.*, *R.*; nana, *R.*; rubiginosa, *Cl.*; tenera, *M.*
Hebeloma crustuliniforme, *Cl.*; sinapizans, *Cl.*, *H.*; versipelle, *Cl.*
Hygrophorus aurantio-splendens Haller, *Ch.*; †brevispora Möller, *Ch.*; calyptraeformis, *Ch.*, *R.*; ceraceus, *Ch.*, *R.*; chlorophanus, *Ch.*, *M.*; chrysaspis Métrod, *M.*; coccineus, *Ch.*, *M.*, *R.*; conicus, *Ch.*, *M.*, *R.*; fornicatus, *Ch.*; hypothejus, *L.*; laetus, *L.*, *R.*; langei, *Cl.*; lepidopus, *Ch.*; pratensis, *Ch.*, *M.*, *R.*; psittacinus, *Ch.*, *L.*, *R.*; puniceus, *Ch.*, *M.*; quietus Kühner, *Ch.*; subminutulus Murrill, *Ch.*; turundus var. mollis, *L.*; unguinosus, *Ch.*, *Cl.*, *L.*, *M.*, *R.*; virgineus, *Ch.*, *Cl.*
Hypholoma capnoides, *Cl.*; fasciculare, *Ch.*, *Cl.*, *L.*, *M.*, *R.*; hydrophilum, *Ch.*, *Cl.*; pyrotrichum, *Ch.*; velutinum, *Ch.*
Inocybe bongardii, *Ch.*; fastigiata, *Cl.*; geophylla, *Cl.*; griseolilacina, *Cl.*; maculata, *Cl.*, *Ch.*, *L.*, *R.*; napipes, *L.*; petiginosa, *Cl.*, *R.*; umbrina, *Cl.*
Laccaria amethystina, *Cl.*, *R.*; laccata, *Ch.*, *Cl.*, *L.*, *R.*
Lactarius blennius, *Ch.*, *Cl.*, *L.*, *R.*; camphoratus, *Ch.*; deliciosus, *R.*; fuliginosus, *R.*; glyciosmus, *Cl.*, *R.*; pallidus, *Ch.*; piperatus, *Cl.*; plumbeus, *Cl.*, *L.*, *R.*; quietus, *Ch.*, *Cl.*, *L.*, *R.*; rufus, *Cl.*, *R.*; serifluus, *R.*; subdulcis, *Cl.*, *L.*, *R.*; tabidus, *L.*
Lepiota acutesquamosa, *Ch.*, *Cl.*; amianthina, *Cl.*, *R.*; cristata, *Cl.*, *M.*; fulvella, *Cl.*; rhacodes, *Cl.*
Leptonia chalybaea, *R.*; lazulina, *Ch.*; sericella, *L.*
Marasmius acervatus *Cl.*, *L.*; confluens, *Ch.*, *Cl.*, *R.*; dryophilus, *Cl.*; oreades, *Ch.*, *Cl.*; peronatus, *Ch.*, *Cl.*, *L.*, *R.*
Mycena acicula, *R.*; ammoniaca, *Ch.*; avenacea, *Cl.*, *R.*; bulbosa, *L.*, *R.*; chlorantha, *M.*; galericulata, *Ch.*, *Cl.*, *M.*, *R.*; galopus, *Ch.*, *Cl.*, *M.*; var. alba, *Cl.*; var. nigra, *Cl.*; speirea, *M.*; stylobates; viscosa, *L.*
Naucoria cucumis, *Ch.*; escharoides, *Cl.*, *R.*; granulosa Lange, *Cl.*
Nolanea infula, *Ch.*; staurospora, *Cl.*, *L.*, *M.*, *R.*
Omphalia fibula, *Cl.*, *L.*; var. swartzii, *Cl.*; hydrogramma, *Cl.*; mairei, *Cl.*; maura, *Cl.*; postii, *Cl.*; umbellifera, *Cl.*
Panaeolus acuminatus auct., *Ch.*, *Cl.*, *M.*, *R.*; ?campanulatus, *Cl.*; papilionaceus, *Ch.*; sphinctrinus, *Ch.*, *R.*
Paxillus involutus, *Cl.*, *L.*, *R.*; panuoides, *Ch.*, *L.*
Pholiota erebia, *Cl.*; lucifera, *Ch.*; mutabilis, *Cl.*, *L.*; spectabilis, *Ch.*
Pleurotus cornucopiae, *Cl.*; petaloides var. geogenius, *Ch.*; rhacodium Berk. & Curtis.
Pluteus cervinus, *Ch.*, *Cl.*, *L.*, *R.*; hiatulus Romagn., *Cl.*, *M.*; salicinus, *R.*
Psalliota amethystina, *Cl.*; arvensis, *Cl.*; silvatica, *Cl.*
Psathyrella disseminata, *Ch.*; fusca, *Cl.*; gossypina, *Ch.*, *L.*, *R.*; gracilis, *Ch.*, *M.*, *R.*

Psilocybe atrorufa, *Cl.*; elongata, *L.*; semi-lanceata, *Ch., L.*
Russula aeruginea, *Ch., L.*; atro-purpurea, *Ch., Cl., L., R.*; chloroides, *Cl.*; cyanoxantha, *Ch., Cl., R.*; emetica, *Ch., Cl., L., R.*; farinipes, *Ch.*; fellea, *Ch., Cl., L.*; foetens, *Cl.*; fragilis, *Cl.*; grisea, *Ch., Cl.*; laurocerasi, *Cl.*; lutea, *R.*; nigricans, *Ch., Cl.*; ochroleuca, *Ch., Cl., L., R.*; pectinata, *Ch., Cl.*; rosea, *R.*; sororia, *Cl.*; venosa, *Cl., L.*; xerampelina, *Cl.*
Stropharia aeruginosa, *Ch., Cl.*; semi-globata, *Ch., L., R.*; squamosa, *Ch.*
Tricholoma columbetta, *Cl.*; loricatum, *Ch., Cl.*; melaleucum, *Ch., Cl., M.*; nudum, *Cl.*; panaeolum, *Ch.*; rutilans, *Ch., Cl., L.*; saponaceum, *Ch., M., R.*; scalpturatum, *Cl.*; sulphureum, *Cl.*; terreum, *Ch., M., R.*; ustale, *R.*
Tubaria autochthona, *M.*; conspersa sensu Romagn., *R.*; crobula, *Cl.*; furfuracea sensu Lange, *Ch., M.*; inquilina, *L.*

HOMOBASIDIOMYCETES—Gasteromycetes

[1]Bovista plumbea, *Cl.*
[1]Calvatia excipuliformis (Pers.) Perdeck, *Cl.*
[1]Crucibulum levis (DC.) Kambly & Lee (syn. C. vulgare Tul.), *Cl.*
Lycoperdon hyemale Bull. em. Vitt., *Cl.*; [1]perlatum var. nigrescens Pers., *Cl., L.*; [1]perlatum Pers. var. perlatum, *Ch., Cl.*; pyriforme, *H.*; [1]spadiceum, *Cl.*
Mutinus caninus, *Ch., Cl.*
[1]Nidularia denudata Fr., *Ch., Cl., L., R.*; [1]minutissima Palmer, *L., R.*
Phallus impudicus, *Ch., Cl., L., R.*
[1]Scleroderma aurantium, *Ch., Cl., L., R.*; [1]bovista, *Cl.*
[1]Sphaerobolus stellatus, *Ch., Cl., L., R.*

FUNGI IMPERFECTI

Hyphomycetes

Acladium conspersum Link, on rotten wood, *L.*
Alatospora acuminata Ing., *Ch., L., M., R.*
Anguillospora longissima Ing., *R.*
Articulospora tetracladia Ing., *Ch., L., R.*
Bispora antennata (Fr.) Mason, on rotten wood, *Ch.*
Brachysporium apicale, on rotten wood, *Cl.*; bloxami, on Quercus, *Ch.*; obovatum, on rotten wood, *Ch.*
Dactylella aquatica (Ing.) Ranzoni, *L.*
Dendrospora erecta Ing., *Ch., L., R.*
Diplococcium spicatum, on rotten wood, *L.*
Flagellospora curvula Ing., *L., M.*
Graphium calicioides, on *Acer*, *M.*
Heliscus aquaticus Ing., *L., R.*; longibrachiatus Ing., *M., R.*; stellatus Ing. & Cox, *R.*
Lemonniera aquatica de Wild, *M.*
Menispora ciliata, on rotten wood, *Cl.*
Monotospora sphaerocephala, on Acer, *M.*
Nectriopsis aureonitens (Tul.) Maire, on rotten wood, *Ch.*
Oidiodendron griseum Robak, on rotten wood, *Ch.*
Oidium aureum Fr., on rotten wood, *Ch.*
Tetracladium marchalianum, *M.*; maxilliformis (Rostrup) Ing., *M.*; setigerum (Grove) Ing., *M.*
Trichothecium roseum, on *Acer*, *Ch., L.*
Tricladium splendens Ing., *L.*
Varicosporium elodeae Kegel, *L., R.*
Verticillium cyclosporum (Grove) Mason & Hughes on *Acer*, *Cl.*; sulphurellum Sacc., on *Quercus*, *Cl.*

AUTUMN DAY FORAYS, 1956

Forays were held as follows: 22 *September*, Windsor Forest; 29 *September*, High Wycombe (joint foray with South Bucks Natural History Society); 6 *October*, Virginia Water; 13 *October*, Juniper Hall; 20 *October*, Burnham Beeches; 28 *October*, Oxshott.

There are no outstanding finds to report.

The number of members attending varied from about twenty at Oxshott to over forty at Virginia Water. The latter is usually the most popular Foray; could this be due to the excellent tea that Dr Topping, Dr Madge and their students are good enough to provide each year?

S. M. FRANCIS
Foray Secretary

LIST OF ALPINE FUNGI PRESENTED TO THE SOCIETY BY DR E. MÜLLER

A collection of 100 Alpine fungi was presented to the Society during the Autumn foray by Dr E. Müller of Zürich. The Council decided that the specimens should be deposited in the general collection of the Herbarium, Royal Botanic Gardens, Kew. The specimens are listed below:

Arnaudiella coronae; Bostrychonema alpestre; Cainia graminis; C. incarcerata; Clathrospora elynae; Dangeardiella aspidii; Diachora onobrychidis; Didymella alectorolophi; D. astragalina; D. eupyrena; D. lophospora; D. nigra; D. obstruens; D. phacae; D. valerianae; D. vincetoxici; Dimeriella oreophila; Dothiopeltis arunci; Dubenia purpurascens; Eupropolella vaccinii; Gibbera artii; G. conferta; G. niesslii; G. petrakii; G. rosae; G. salisburgensis; G. vaccinii; Gnomonia geranii; Guignardia bulgarica; G. cytisi; G. epilobii; G. foeniculata; G. gentianicola; G. latemarensis; G. rosae; G. tofieldiae; G. uncinata; Isothea rhytismoides; Laetinaevia epilobii; L. jenensis; Lasiobotrys lonicerae; Leptoguignardia onobrychidis; Leptopeltis aquilina; Leptopeltopsis nebulosa; Leptosphaeria anemones; L. centaureae; L. cornuta; L. derasa; L. epilobii; L. helminthospora; L. heterospora; L. johansoni; L. ladina; L. primulaecola; L. robusta; L. septemcellulata; L. silenes-acaulis; L. silvatica; L. trollii; L. volkartiana; Mamianiella coryli; Ophiobolus clavatus; O. fruticum; O. mayori; O. olivaceus; O. tanaceti; O. volkartii; Phacidium pini-cembrae; Phyllachora therophila; Physalospora rhodendendri; Pirottea geranicola; P. longisetosa; Plectosphaera spartii; Pleospora anthyllidis; P. brachyspora; P. caudata; P. pileata; P. planispora; P. potentillae; P. pyrenaica; P. raetica; P. sieversiae; P. subalpina; P. valesiaca; P. myrtillina; Plowrightia mezerei; Pseudopeziza pedicularis; Pyrenophora phaeocomes; Seynesiella juniperi; Venturia alchemillae; V. anemones; V. atriseda; V. centaureae; V. cephalariae; V. epilobii; V. lonicerae; V. polygoni-vivipari; V. rumicis; Wettsteinina dryadis; W. engadinensis; W. mirabilis; W. pachyasca.

ANNUAL GENERAL MEETING

Saturday, 8 December 1956. The sixtieth Annual General Meeting was held in the rooms of the Linnean Society of London at 12 noon, with the President, Mr W. D. Graddon, in the Chair.

After the Minutes of the previous Annual General Meeting had been read and signed, the President recorded with regret the deaths during the year of Dr C. L. Shear and former members H. N. Ridley and Owen Whitaker. He then reported briefly on the year's activities.

The Treasurer presented his Annual Statement which was accepted *nem. con.*

The following Officers and Council were then elected for 1957:

President: C. J. Hickman.

Vice-President: L. Ogilvie, in addition to past Presidents, Miss L. E. Hawker and W. D. Graddon.

Treasurer: W. Buddin.

Secretary: J. Webster.

Foray Secretary: Mrs S. M. Francis.

Plant Pathology Committee Secretary: J. M. Hirst.

Editors: G. C. Ainsworth, S. D. Garrett.

New Members of Council: C. T. Ingold, D. M. Henderson, R. V. Harris (to replace J. Colhoun, J. G. Manners, B. E. Plunkett).

New Members of Plant Pathology Committee: R. J. W. Byrde, F. C. Deighton, Miss J. Ives.

New Members of Foray Committee: Miss G. M. Waterhouse, J. G. Manners.

Prof. J. A. Nannfeldt was unanimously elected an Honorary Member of the Society in recognition of his pre-eminence in mycology.

Twelve new members were then elected, making a total of forty-five new members during the year.

At 2.30 p.m. the President gave an address entitled 'Approach to Discomycetes' (see these *Transactions*, **40**, 1–8, 1957). The meeting concluded with a vote of thanks to the President, proposed by Dr L. E. Hawker and seconded by Dr J. G. Manners.

RECEIPTS AND PAYMENTS for the year ended 30 June 1956

RECEIPTS

	£ s. d.	£ s. d.
1 July 1955, Balance brought forward ...		2884 13 8
Cambridge University Press Sales:		
Transactions, Vol. 38	991 10 6	
Reprints	316 12 3	
Back Volumes	104 12 1	
50-year *Index* and *Lichen Catalogue*	19 1 1	
Miscellaneous	11 15 2	
List of Common Brit. Plant Diseases	3 16 6	
		1447 7 7
Sundry Sales at Meetings, etc.		9 19 4
Subscriptions:		
Arrears	3 0 0	
Current	839 14 8	
In advance	21 7 9	
Associates	26 6 0	
Repayment of Income Tax	83 14 2	
		974 2 7
Donations:		
Cheesman and Pethybridge Funds ...	3 10 0	
Plates and Printing Funds	11 5 0	
Hospitality Fund	4 6 3	
Library	8 4 10	
		27 6 1
Grants, etc.:		
Through Royal Society (1955)	300 0 0	
A. A. Pearson Bequest (2nd moi. and int.)	101 7 4	
		401 7 4
Interest:		
Capital Reserve Fund	35 4 7	
P.O. Savings Bank and Deposit Account	51 18 1	
		87 2 8
		£5831 19 3

PAYMENTS

	£ s. d.	£ s. d.
Congratulatory Addresses		3 19 0
Cambridge University Press:		
Production *Transactions*, Vol. 38	1508 6 11	
Reprints	169 3 0	
Distribution of Members' Copies, etc. ...	52 17 4	
Commissions on:		
Production (Vol. 38 and Reprints) ...	216 5 11	
Members' Copies	58 10 6	
Sales of Vols. 1–38 and Misc. ...	142 4 5	
Sales of *Index* and *Lichen Catalogue* ...	4 15 3	
		2152 3 4
Library Insurance		1 16 0
Purchase of 4% Defence Bonds		500 0 0
Sundry Printing		60 7 5
Postages, Stationery, etc.		64 7 2
Fees for Meetings		7 7 5
Subscriptions:		
Woolhope Club	1 0 0	
Brit. Committee for Int. Nat. Cons. (2 year)	4 0 0	
Field Studies Council	2 2 0	
		7 2 0
Grants on Cheesman and Pethybridge Funds		8 8 0
Grants on Hospitality Fund		15 7 9
Balance:		
At Midland Bank (inc. Deposit)	877 10 2	
In Post Office Savings Bank	2133 11 0	
		3011 1 2
		£5831 19 3

CAPITAL RESERVE FUND (Nominal Values of Stock)

Cheesman & Pethybridge Funds ... £260 of 3½% War Stock
Lister Fund & Pearson Bequest ... £500 of Saving Certificates

General, Hospitality, Library and Printing Funds ... ... £365 of 3½% War Stock
£550 of Saving Certificates
£200 of 3½% Defence Bonds
£500 of 4% Defence Bonds

GRAND TOTAL ... £2375

Examined and found correct (*Signed*) W. C. MOORE; (*Signed*) WALTER BUDDIN, *Hon. Treasurer.*

Donations and Grants to the several Funds of the Society are gratefully acknowledged.

REVIEWS

Soil Zoology. Edited by D. K. McE. Kevan. (London: Butterworths Scientific Publications. 1955.) Pp. xiv + 512. Price 55*s*.

This book gives an account of the Proceedings of the University of Nottingham Second Easter School in Agricultural Science, 1955. It is a stimulating compilation, bearing out the contention that the importance of animals in soil processes has been seriously underestimated, and particularly deserves the attention of soil microbiologists. The first part is devoted to papers and discussion, and the (smaller) second part to methods of which practical demonstrations were given. Introductory papers are concerned with the study and identification of soil animals and accounts are also given of selected groups, the nematodes, mites and termites. The activity of termites in relation to structure and fertility of tropical soils is of particular interest, and there is mention of the status of fungi 'cultivated' in termitaria. In the section on pedology there is an outstanding paper by Kubiena on the relation of animal activity to humus forms, illustrated by beautiful photomicrographs of thin humus sections. There are further sections dealing with a variety of ecological and applied problems, contributions by Tischler about the influence of soil type and cultural practice on the fauna of agricultural soils being especially instructive. A small final section is concerned with fungi attacking nematodes and protozoa. The second part of the book contains short accounts of two methods interesting to students of the soil microflora, an agar-embedding technique for studying moist, undisturbed soil and a resin-impregnating technique for preparing permanent sections. The variety of approach and the emphasis on field work shown by current workers in this field of zoology constitutes a striking challenge to soil mycologists and bacteriologists. With certain important exceptions it may well be true, as Kubiena maintains, that the latter's highest achievements have been reached in the investigation of organisms isolated from the soil and that the study of real soil microbiology hardly exists.

J. RISHBETH

Mildious, Oidiums, Caries, Charbons, Rouilles des Plantes de France. By G. Viennot-Bourgin. (*Encyclopédie Mycologique*, xxvi, xxvii. Paris: Paul Lechevalier. 1956.) *Texte*, vi + 317 pp.; *Atlas*, 89 plates. Price (the 2 vols.) 18,000 French francs.

The first of these two quarto volumes comprises a list of the downy mildews, powdery mildews, smuts and rusts of the cultivated and wild plants of France, together with keys to the genera and an index to the species of the parasites and a glossary of mycological terms. In the second, host symptoms and microscopic details of the pathogens (based on studies by the author) are illustrated by black and white drawings.

The fungi are arranged under the generic names of the hosts which are listed alphabetically from *Abies* (p. 15) to *Zea* (p. 296). For each genus the species are also listed alphabetically and the diagnostic features of the fungi are briefly indicated (as dichotomous keys when more than one species of any of the four groups is recorded for one host genus). Similarly, in the Atlas, after a series of eight plates illustrating the fungal genera, the illustrations of the species of fungi are arranged according to the host genera.

In these days of austerity in the publication of scientific data it may seem somewhat ungenerous to complain at the lavish format, but the information in the first volume could have been presented more compactly and the wisdom of arranging the illustrations under the hosts is a matter of opinion. One feature on which there will be no disagreement is the excellence of the plates. Prof. Viennot-Bourgin's skill as a draughtsman is well known and the Atlas will surely find a place as an outstanding example of mycological art.

G. C. AINSWORTH

[These two volumes have been placed in the Society's Library.]

Cryptococcosis. Torulosis or European Blastomycosis. By M. L. LITTMAN and L. E. ZIMMERMAN. (New York: Grune and Stratton. 1956.) x + 205 pp., 4 coloured plates, 82 figures. Price U.S. $8.50.

Twenty-five years ago cryptococcosis (caused by *Cryptococcus neoformans*) was a medical curiosity. To-day it is being recorded with increasing frequency from all parts of the world. There is, however, no reason to suppose that there has been any marked increase in the incidence of cryptococcosis during this period, and the frequency with which this usually serious and often fatal disease of man and animals is diagnosed seems to depend directly on the incidence of medical mycologists. This book thus reflects the increasing attention paid to medical mycology during recent years. It is also a model of its kind and merits emulation by both medical mycologists and plant pathologists. Few fungus diseases have received such satisfactory monographic treatment. All aspects of the disease are concisely dealt with in a manner which will satisfy adequately, if not completely, the clinician, the pathologist, and the mycologist. The text is well but unobtrusively documented (there are 504 references cited in the bibliography) while the illustrations, whether in colour or half-tone, are informative and of excellent quality. If this standard is maintained in the further monographs on mycoses of man which are promised from the Armed Forces Institute of Pathology (Washington, D.C.) they will be eagerly awaited.

G. C. AINSWORTH

Losses caused by Plant Diseases in the Colonies. By G. WATTS PADWICK (Phytopathological Papers, No. 1, Commonwealth Mycologica Institute, Kew, 1956.) Pp. 60, 10 plates, 7 text-figures. Price: 10*s*

This publication should be of special interest to members of the British Mycological Society, because in 1941, a Subcommittee of the Plant Pathology Committee was set up to foster research into, and ultimately to report upon, methods of plant disease assessment. The Subcommittee recognized that the development of such methods was a research problem in itself, and was best investigated by those plant pathologists already working upon the individual diseases for which a method of assessment was sought. The Subcommittee encouraged and assisted these workers to produce methods of disease assessment usable by the average plant pathologist (see W. C. Moore, *Trans. Brit. mycol. Soc.* **26**, 28–35, 1943).

The position in the Colonies is very different, inasmuch as disease problems of major economic importance vastly outnumber the experienced plant pathologists that are available. It is necessary to name only a few—swollen shoot of cacao, Panama disease of bananas, sudden death of cloves; these and others have influenced and are influencing the course of colonial history. In his attempt to assess the effect of these diseases upon the economy of the territories concerned, Dr Watts Padwick has essayed a task that precise-minded persons find inherently distasteful; he has amassed a great volume of information widely scattered in time and space of collection, undeterred by his confessed recognition of the fact that most of these data are far from precise. Thus he comments on p. 24: 'It must be recorded here that the percentage losses can at best be regarded as only rough estimates. The individual data from which they are built up are based more often than not on general impressions of a local plant pathologist, which, though better than nothing, are obviously far from accurate.' And, on p. 30, he remarks: 'No statistical treatment, however skilfully contrived, can make good the short-comings of faulty or inadequate observation.' This difficulty Dr Watts Padwick has got over, in his 23-page appendix of crop losses due to various diseases, by classifying losses under the very broad, and therefore reasonably reliable, categories of the Colonial Plant Disease Survey (A, more than 10% loss; B, important losses under 10%; C, small or very small losses, assumed to average 1%).

Dr Watts Padwick has had extensive first-hand experience of plant pathology in tropical territories, and so he has produced much more than a mere library compilation; his authoritative account of this subject is certain to be widely quoted for years to come.

S. D. GARRETT

THE BRITISH MYCOLOGICAL SOCIETY
TRANSACTIONS

Vol.	PART 1	2	3	4	5
1	*m*	*m*	6*s.*	*m*	*m*
2	(		68*s.*		)
3	(		80*s.*		)
4	*m*	*m*	—	—	—
5	*m*	*m*	*m*	—	—
6	*m*	*m*	(20*s.*	)	—
7	(		40*s.*	)	—
8	(		40*s.*	)	—
9	(	20*s.*	)	*m*	*m* —
10	(		40*s.*	)	—
11	(		40*s.*	)	—
12	(10*s.*)	*m*	*m*	(10*s.*)	—

Vol.	PART 1	2	3	4
13	*m*	*m*	*m*	*m*
14	(		40*s.*	)
15–16	*m*	*m*	*m*	*m*
17	*m*	*m*	(10*s.*)	*m*
18	*m*	(	30*s.*	)
19	*m*	(	30*s.*	)
20–29	(		40*s.*	)
30	(	Jubilee Vol. 30*s.*		)
31	[	30*s.*	] *m*	*m*
32	[	30*s.*	] *m*	*m*
33		[	60*s.*	]
34–39	(		60*s.*	)

m = out of print
( ) = volume (or part volume) sold separately
[ ] = only sold as part of a run of volumes

REPRINTS AND MISCELLANEOUS PUBLICATIONS

PLANT PATHOLOGY COMMITTEE: *List of Common British Plant Diseases*, 3rd Ed. 1945. Price net, 5*s.* Postage, 4*d.*

T. PETCH: *British Hypocreales. Ex* Vol. 21, Pts. 3 & 4. 1938. Price net, 2*s.* Postage, 3*d.*

A. A. PEARSON & R. W. G. DENNIS: *Revised list of British Agarics and Boleti. Ex* Vol. 31, Pts. 3 & 4. 1948. Price net, 5*s.* Postage, 3*d.*

J. RAMSBOTTOM & F. L. BALFOUR-BROWNE: *List of Discomycetes recorded from the British Isles. Ex* Vol. 34, Pt. 1. 1951. Price net, 5*s.* Postage, 3*d.*

FIFTY YEAR INDEX TO THE TRANSACTIONS, Vols. 1–30; 1896–1946. Compiled by G. R. BISBY. Price net, 21*s.* Postage, 6*d.*

E. M. BLACKWELL: Haustoria of *Phytophthora infestans* and some other species. *Ex* Vol. 36, Pt. 2. 1953. Price 4*s.* Postage, 3*d.*

K. SAMPSON & J. H. WESTERN: *Diseases of British Grasses and Herbage Legumes.* 2nd Edition. 1954. Price 15*s.* Postage, 6*d.*

HILDA M. CANTER: *Annotated List of British Aquatic Chytrids. Ex* Vol. 36, No. 4. 1953. Price 3*s.* Postage, 2*d.*

MALCOLM WILSON & G. R. BISBY: *List of British Uredinales. Ex* Vol. 37, No. 1. 1954. Price 3*s.* Postage, 2*d.*

AGNES H. S. BROWN & GEORGE SMITH. *The genus* Paecilomyces *Bainer and its perfect stage* Byssochlamys *Westling. Ex* Vol. 40, No. 1. 1957. Price 12*s.* 6*d.* Postage, 3*d.*

Enquiries and orders for the above to be addressed to

CAMBRIDGE UNIVERSITY PRESS
200 EUSTON ROAD, LONDON, N.W. 1

or to

CAMBRIDGE UNIVERSITY PRESS
AMERICAN BRANCH, 32 EAST 57TH STREET, NEW YORK 22

FORAY COMMITTEE PUBLICATIONS

(*To be obtained from the Treasurer and on sale at meetings and forays*)

A key to the genera of the agarics and Boleti. By A. A. PEARSON. 32 pp. 1950. (Out of print. New edition in preparation.)

Guide to the literature for the identification of British fungi. 1952. Price 2*s.* 6*d.* Postage, 2*d.*

Census Catalogue of British Lichens. By W. WATSON. 1953. Price 7*s.* 6*d.* Postage, 4*d.*

CONTENTS

PAGE

Pleospora straminis, P. rubelloides and *P. rubicunda*: three fungi causing purple staining of decaying tissues. By John Webster. (With 3 Text-figures) 177

Penicillium isariaeforme sp.nov. and *Aspergillus raperi* sp.nov. By Amelia C. Stolk and J. Meyer. (With Plate 8 and 2 Text-figures) 187

New or interesting records of British Ustilaginales. By Derek A. Reid. (With 8 Text-figures) 193

The taxonomy of *Stereum traplianum* Velenovsky. By Derek A. Reid. (With 1 Text-figure) 200

Studies on British species of *Endogone*. III. Germination of spores. By Rosemary M. Godfrey. (With 1 Text-figure) 203

Hanseniaspora vineae sp.nov. By J. P. van der Walt and Irmgard T. Tscheuschner. (With 1 Text-figure) 211

Sporophore production by *Polyporus arcularius* Batsch ex Fr. in culture. By I. A. S. Gibson and Jennifer Trapnell 213

Factors affecting the germination of sclerotia and mycelial growth of *Sclerotium rolfsii* Sacc. By D. V. W. Abeygunawardena and R. K. S. Wood . . . 221

Occurrence of *Ramulaspera holci-lanati* in Britain. By Ellis Griffiths. (With Plate 9 and 3 Text-figures) 232

Studies on the occurrence and activity of fungi in a wheat-field soil. By J. H. Warcup. (With Plate 10 and 3 Text-figures) 237

A *Cercospora* leaf spot of ramie in Assam. By S. Chowdhury. (With 1 Text-figure) . 260

Notes on British agarics 1–5 (Observations on the genus *Coprinus*). By Peter D. Orton. (With 9 Text-figures) 263

Thielavia indica sp.nov. By S. B. Chattopadhyay and C. Das Gupta. (With 1 Text-figure) 277

Observations on Gasteromycetes, 5. Studies on type material no. 3, *Geastrum recolligens* (Woodw. ex Sow.) S. F. Gray. By J. T. Palmer. (With 1 Text-figure) . . 279

Behaviour of soil fungi in the presence of bacterial antagonists. By David Park . 283

On the marine phycomycete *Thraustochytrium proliferum*. By T. W. Johnson, Jr. (With 1 Text-figure) 292

Proceedings 000

The Sheffield Foray 295

Autumn Day Forays, 1956 300

List of Alpine fungi presented to the Society by Dr E. Müller 300

Annual General Meeting 301

Receipts and Payments, 1956 302

Reviews 303

INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and subheadings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, The Royal Society, Burlington House, London, W. 1, price 2*s*. 6*d*.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

The British Mycological Society subscribes to the Fair Copying Declaration, details of which may be obtained from the offices of the Royal Society.

Printed in Great Britain at the University Press, Cambridge (*Brooke Crutchley, University Printer*)